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Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those who have made substantial contributions to the study.

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2- Books:

(a) Personal author: Speroff L, Glass RH, Kase NO. clinical gynecologic endocrinology and infertility. 4th edition, Baltimore, Williams & Wilkins; 1988: 105

(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum 1, Hamberger L. Interaction between prostaglan dins and catecholamines on cervical collagen. In: Toppozada M., Bygdeman '. M., Hafez ESE, Eds. Prostaglandins and fertility regulation. Advances in reproductive health care. Lancaster, England, MTP Press Ltd., 1985 : 75 - 80.

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Contents :

Letter from the Editor:

Dear colleagues,

very interesting subjects are included in this edition. When a woman presents with preterm pre-labor rupture of the membranes, it is recommended to employ either transvaginal cervical length, amniotic fluid index, or both to forecast delivery delay. Early premature deliveries make PPROM management difficult. Variations in medical procedures need customized care. NICU-admitted newborns have poor neonatal outcomes, requiring tailored care and outcomes initiatives.

To reduce the likelihood of Ovarian Hyperstimulation Syndrome (OHSS) occurring, certain measures can be taken, including employing the GnRH antagonist protocol for inhibiting the pituitary gland and stimulating ovulation through the use of a GnRH agonist, as well as cryopreservation of all embryos (IVF/ICSI cycle segmentation). Close monitoring of PCOS patients during IVF/ICSI with treatment plans individualization.

Administration of prophylactic systemic antibiotic post episiotomy is not effective to prevent wound infection. The endometrial volume changes after progesterone administration was the only significant independent predictor of clinical pregnancy rate in frozen embryo transfer (FET) cycles. Furthermore, a change in the endometrial volume of 10.44% was associated with significant improvement in clinical pregnancy rates of FET cycles with artificial endometrial preparation.

Chromohysteroscopy appears to improve the efficacy of hysteroscopy in abnormal uterine bleeding and observation of diffuse light blue staining without dark areas strongly suggests a normal endometrium free of endometritis. The effectiveness of intraperitoneal drainage in reducing post-laparoscopic shoulder pain during the first 24 hours after surgery, consequently reducing the need for postoperative analgesics. These findings support the outcomes of previous investigations, indicating that drain placement may be a valuable strategy to alleviate postoperative shoulder pain in women undergoing gynecologic laparoscopy.

Best regards.

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Accuracy of Fetal Thigh Circumference and Fractional thigh Volume in prediction of Fetal Birth Weight

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Abstract

Background: Accurately estimating fetal weight facilitates optimal care planning. Traditionally, formulas using biometric parameters like the biparietal diameter (BPD) and femur length (FL) guided assessments. More recently, ultrasound measurements of the fetal thigh circumference and fractional thigh volume emerged as promising alternative predictors.

Aim: To evaluate fetal thigh circumference & fractional thigh volume's accuracy in predicting birth weight.

Methods: We recruited 60 women in their third trimester with singleton pregnancies, excluding cases with anomalies or gestational age discrepancies. Standardized ultrasound biometry between 37-40 weeks measured parameters including BPD, abdominal circumference (AC), head circumference (HC), FL, thigh circumference (TC) & thigh volume (TVol). Estimates utilized Hadlock's, Vintzileos', Lee formulas. Actual weights were recorded post-delivery.

Results: The average age of participants was 29.38 ± 7.56 years. Gestational age varied among 37& 40.29 weeks. Actual birth weight mean was 3552.60 ± 689.87 grams. Estimated weights: Hadlock's - 3366.38 ± 590.82 grams, $p<0.001$; Vintzileos' - 3299.60 ± 630.95 grams, $p=0.003$; Lee 1 formula - 3693.07 ± 778.83 grams, $p<0.001$; Lee 2 - 3596.52 ± 754.27 grams, $p=0.012$. No significant variance among estimated & actual TC (pgreater than0.05). Strong positive correlations between actual weight and all estimates ($p<0.001$), highest for Lee 2 ($r=0.985$) and Lee 1 formula ($r=0.984$). Multiple regression identified Lee 1 and Lee 2 formula as significant predictors ($p=0.040$, 0.002). ROC analysis found optimal sensitivity/specificity for TVol (100/95.8%), Hadlock's (100/93.7%), Lee 1 formula (100/95.8%), Lee 2 (100/95.8%) but reduced for Vintzileos' (75/87.5%).

Conclusion: Our study revealed that Lee 2 formula proved most clinically reliable. TC and TVol also strongly correlated with actual weight, indicating reliability. TVol exhibited high diagnostic value.

Keywords: fetal weight estimation, thigh circumference, fractional thigh volume, gestational age, predictive accuracy.

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Introduction

Accurate predicting the fetus birth weight is vital for the early identification of potential complications & the facilitation of appropriate interventions. Various methods exist for estimating fetus birth weight, with notable techniques including the measurement of fetus thigh circumference and fractional thigh volume [1].

Fetal thigh circumference, which involves the ultrasonic measurement of the thickness of the fetus thigh at a specific gestational age, has been proposed as a reliable indicator of fetus growth and subsequent birth weight. This method employs ultrasound technology to determine the diameter of the fetus thigh, subsequently utilizing these measurements to estimate the fetus weight [2].

Similarly, fractional thigh volume offers another non-invasive approach for estimating fetus birth weight. This technique also relies on ultrasound to evaluate the volume of the fetus thigh, which is then used to predict fetus weight. An advantage of this method is its capacity for repeated assessments over time, allowing for continuous monitoring of fetus growth [3].

Both methods have been explored as potential predictors of fetus birth weight. Nevertheless, the accuracy of fetus thigh circumference and fractional thigh volume can vary significantly across different studies and populations [4]. Factors such as gestational age notably influence their reliability. Research indicates that these methods tend to be more accurate later in pregnancy when the fetus is more fully developed [5].

Despite potential challenges, fetus thigh circumference and fractional thigh volume remain valuable tools for estimating fetus birth weight in low-risk pregnancies. It is crucial, however, to recognize their limitations and to integrate them with other diagnostic approaches like fetus biometry to enhance the accuracy of birth weight estimations [6].

While promising, fetus thigh circumference and fractional thigh volume require further investigation to fully ascertain their utility and constraints. It is important to account for variables such as gestational age and the presence of fetus anomalies when employing these methods to predict fetus birth weight [7].

This research aims to evaluate fetus thigh circumference accuracy & fractional thigh volume as predictors of fetus birth weight, with a focus on understanding the influences of gestational age and fetus development patterns on these predictive tools.

Patients and methods

Study Design & Setting

This cross-sectional research was performed among January 2023 & October 2023 at Menoufia University Hospital and Shebeen El-Kom Teaching Hospital.

Participants

We recruited 60 pregnant women from the outpatient obstetrics & gynecology clinics of the 2 hospitals. Participants were included if they were in the third trimester of pregnancy with a single, viable fetus. We excluded women with multiple gestations, pregnancies complicated by major congenital anomalies, or a gestational age discrepancy exceeding four weeks as determined by their last menstrual period relative to ultrasound findings.

Ethical Considerations

Prior to information gathering, ethical approval was obtained from the Institutional Review Board of Menoufia University, Faculty of Medicine. informed consent was secured from each participant, emphasizing the voluntary nature of their involvement & the confidentiality of their data through anonymization and secure handling.

Data Collection

Data collection began with comprehensive history taking, covering demographic details,

personal and family medical history, and detailed obstetric and menstrual histories. This was followed by a thorough physical examination which included assessment of vital signs and visible health indicators (pallor, cyanosis, jaundice, lymph node enlargement).

Ultrasound Imaging Protocol

Fetus biometry was performed using a standardized ultrasound protocol with a 2-D transabdominal approach using GE LOGIQ P5, United States ultrasound machine with a 3.5 MHz abdominal probe between thirty-seven & forty weeks of pregnancy. Measurements included BPD, HC, AC, FL, TC & TVol.

Estimation of Fetal Weight

Fetus weight estimations were derived using these formulas:

- Hadlock's formula, which doesn't utilize TC as a parameter. The formula is as follows:
- Log (Expected fetus weight) = 1.487-0.003343 × AC × FL + 0.001837 × BPD × BPD + 0.0458 × AC + 0.158 × FL [8].
- Vintzileos' formula, which uses the fetus thigh circumference as a parameter. The formula is as follows:
- Log (Birth weight) = 1.897 + 0.015 × AC + 0.057 × BPD + 0.054 × FL + 0.011 × TC [9].
- Lee formulas which used only the (Tvol):

- Lee 1: EFW = e^(4.708 + 0.7596 × ln(TVol)) [10].

- Lee 2: ln weight = − 0.8297 + 4.0344 (ln BPD) − 0.7820 (ln BPD)2 +0.7853 (ln AC) + 0.0528 (ln TVol)2 [11].

Follow-up and Measurement Validation

Participants were monitored through to delivery. For those not delivering within a week post the last scan, repeat measurements were taken. Newborns were weighed within half an hour of birth, using a calibrated neonatal scale, and thigh circumferences were measured manually to validate ultrasound estimates.

Statistical Analysis

Data handling and analysis were conducted utilizing SPSS version 26.0 & Microsoft Excel 2016. Quantitative information were explored for normality & analyzed accordingly using mean ± SD for parametric and median with interquartile ranges for non-parametric distributions. The inferential statistical methods included: Independent t-tests or Mann-Whitney U tests to compare two independent samples, paired t-tests for comparisons within the same group and pearson correlation analysis for assessing relationships between continuous variables. ROC curve analysis to determine the diagnostic accuracy of ultrasound estimates against actual neonatal weights. Significance thresholds were set at less than 0.05 for significant outcomes.

Results

The research was carried out on 60 pregnant women with single viable fetus. The age of studied women varied among 18 & 40 years with mean ±SD was 29.38± 7.56 years. The gestational age ranged from 37 to 40.29 weeks with mean ±SD was 38.58± 1.09 weeks. The mean BMI in studied cases was 26.32±2.99 Kg/m2. More than half women (61.7%) were multipara and more than half of them (61.7%) were from rural areas (Table 1).

The mean biparietal diameter (BPD) was 10.77±11.00 mm, mean head circumference was 33.03 ±1.97 mm, mean abdominal circumference (AC) was 34.10±2.40 mm, mean estimated thigh circumference was 16.17±1.66 mm while mean femur length (FL) was 7.20±0.36 mm. Regarding actual thigh circumference, it had a mean of 16.27 ±1.69 mm.

There is a significant variance among real birth weight & estimated fetus weight by Hadlock's method (Pless than 0.001) with mean difference of 186.22 grams. Also, there is a significant variance among real birth weight & estimated fetus weight by

Vintzileos' method (P=0.003) with mean difference of 253 grams. In addition, there is a significant variance among real birth weight & estimated fetus weight by Lee 1 formula (P<0.001) with mean difference of 140.47 grams. In addition, there is a significant variance among real birth weight & estimated fetus weight by Lee 2 method (P=0.012) with mean difference of 43.92 grams (Table 2).

The there is no statistically significant variance among estimated thigh circumference by ultrasound & actual thigh circumference of the studied cases (P >0.05).

There was a significant positive association among real birth weight with estimated fetus weight by Hadlock's method (r=0.967, p less than 0.001), estimated fetus weight by Vintzileos' method (r=0.934, p<0.001), estimated fetus weight by Lee 1 formula (r=0.984, p less than 0.001), estimated fetus weight by Lee 2method (r=0.985, p<0.001), and Tvol (r=0.979, p less than 0.001). Also, there was a significant positive association among real birth weight with head circumference (r=0.936, p less than 0.001), estimated TC (r=0.830, p less than 0.001), femur length (r=0.819, p less than 0.001), & femur length (r=0.819, p less than 0.001) (Table 3).

A multiple regression was run to predict factors predicting fetus birth weight. This resulted in a significant model, (p less than 0.001, R2 = 0.990). The above-mentioned predictors were examined further and indicated that fetus weight estimated by Lee 2method (p= 0.040) and fetus weight by Lee 1 formula (p= 0.002) were significant predictors (Table 4).

ROC examination was conducted to detect the diagnostic value of fetus thigh circumference, fractional thigh volume and estimated fetus weight by different formulas in prediction of fetus birth weight. Actual TC, Tvol, EFW by Hadlock's method and FW by Lee 1 formula had the highest sensitivity. While Tvol, and FW by Lee 1 formula had the highest specificity. We reported that, the sensitivity of test using estimated TC was 91.7%, the specificity was 81.2%, with AUC was 0.867. However, when we use Actual TC predict the fetus weight, we reported that, the sensitivity of the test was 100%, the specificity of 81.2% with AUC was 0.941. Tvol predict the fetus weight with sensitivity of the test was 100%, the specificity of 95.8% with AUC was 0.987. Detection of birth weight by US using Hadlock's Formula (g), we reported that, the sensitivity of test was 100%, the specificity was 93.7%, with AUC was 0.984. Though, when we use Vintzileos's Formula (g) to predict the fetus weight we reported that, the sensitivity of the test was 75%, the specificity of 87.5% with AUC was 0.814. EFW by Lee 1 formula predict the fetus weight with sensitivity of the test was 100%, the specificity of 95.8% with AUC was 0.987. EFW by Lee 2 Formula predict the fetus weight with sensitivity of the test was 100%, the specificity of 95.8% with AUC was 0.986 (Table 5).

Discussion

Estimates of fetus weight (EFWs) in late pregnancy are critical for obstetric decision-making. While fundal height and gestational age provide a crude estimation, ultrasound biometry offers superior accuracy. Commonly, EFW at 30 weeks predicts term weight using HC, AC, & FL [12].

Fetal TC was recently added as an additional biometric parameter for sonography. In addition to estimating the weight of the fetus at birth, TC is also capable of detecting changes in soft tissue masses. The inclusion of fetal TC along with other sonographic parameters has been shown to provide a more accurate estimation of fetal weight [13].

The main purpose of this research was to determine the accuracy of fetus TC & fractional TVol in predicting fetus birth weight.

In our research, we observed that the ages of the women we studied varied among 18 & 40 years, with an average age of 29.38 +7.56 years. The gestational ages of these women ranged between 37 and 40.29 weeks, with an average of 38.58 +1.09 weeks. A significant proportion, 61.7%, were multiparous, and the same percentage originated from rural areas.

Our findings align with those reported by Ali et al. [14], who investigated the precision of prenatal weight predictions based on fetal TC in a cohort of 123 pregnant women with live singleton term babies. Their study noted that the participants' ages varied among 17 & 39 years, with an average age of 26.68 years and an SD of 5.24 years. They reported gestational ages between 38 and 41 weeks, with an average of 38.78 weeks and an SD of 0.85 weeks, and found that 64.2% of the women were multiparous.

In this study, we recorded an actual mean birth weight of 3552.60 +689.87 grams. This served as the primary benchmark for evaluating the accuracy of various fetus weight estimation methods. Hadlock's method provided a slightly lower average estimate of 3366.38 +590.82 grams, indicating a tendency for conservative estimations. The Vintzileos' method estimated even lower, with a mean of 3299.60 +630.95 grams, further underestimating the actual weight. Conversely, the Lee 1 formula yielded a higher mean estimate of 3693.07 +778.83 grams, suggesting a potential overestimation. The Lee 2 method, however, presented a closer approximation to the real birth weight at an average estimate of 3596.52 +754.27 grams, demonstrating its improved accuracy. The fractional thigh volume (TVol), a separate metric, had a mean value of 102.4 +28.34, highlighting the variety of available metrics for fetal weight estimation.

Our findings are supported by Tahira et al. [7], who associated fetus TC at 36-40 weeks with birth weight, reporting an actual mean weight of 3342.4 +423.74 grams, with Hadlock's and Vintzileos' methods providing estimates of 3319.9 +354.52 grams and 3450.4 +89.68 grams, respectively.

Consistency with our results was also found in the study by Mohamed et al. [15], which reported an actual mean weight of 3204.31 +205.275 grams, with the Hadlock's, Vintzileos', and Lee 1 methods yielding estimates of 3466.35 +210.784 grams, 3244.15 +210.625 grams, and 3333.90 +476.43 grams, respectively. Their reported TVol mean was identical to ours at 102.4 +28.34.

Similarly, Park et al. [16] and Sanyal et al. [9], provided comparative findings with actual mean weights of 3025 +519 grams and 2736.79 +520.43 grams, respectively. These studies explored the efficacy of Hadlock's and Vintzileos' methods alongside TVol, with Park et al. reporting a TVol mean of 122.9 +35.6 and Sanyal et al. showcasing the predictive capabilities of both formulas in assessing fetus weight closely to real birth weight.

Furthermore, Simcox et al. [17], aimed to establish normal values for 3D fractional thigh volume in detecting fetal growth restriction during the third trimester. They found a TVol mean of 90.3, with a range from 59.3 to 121.3, underscoring the potential of 3D measurements in enhancing fetal weight estimation accuracy.

In this study, we discovered no statistically significant variance among the assessed thigh circumference measured by ultrasound & the real TC of the participants (P greater than 0.05). This finding indicates a high degree of accuracy in ultrasound measurements for estimating fetal TC.

Supporting our findings, Mohamed et al. [15], observed a similar lack of statistical significance between assessed TC by ultrasound & real TC, with a P-value of 0.0602. This parallels our results, suggesting consistency in the precision of ultrasound estimations across different cohorts.

Ali et al. [14], also found no statistically significant discrepancy between estimated and actual TC (P = 0.06), echoing the consistency of ultrasound measurement accuracy highlighted in our study and others.

Our analysis revealed statistically significant differences between actual birth weights and estimates from Hadlock's, Vintzileos', Lee 1's and Lee 2's methods. Specifically, Hadlock's underestimated by an average of 186 grams, exhibiting conservative predictions. Vintzileos' showed greater underestimation at 253 grams on average. Conversely, Lee 1's and Lee 2's estimates were closer at average discrepancies of 140 and 44 grams respectively, with Lee 1's potential for overestimation.

Support for our findings comes from Ait-Allah et al. [18], who observed statistically significant differences among the real birth weights & those assessed by Hadlock's method (P less than 0.001) and by Vintzileos' method (P<0.001), resonating with our observations of these methods' tendencies for underestimation.

Similar concordance with our outcomes was documented by Sanyal et al. [9], who detect significant differences among actual birth weights & the estimates by Hadlock's method (P<0.001) and Vintzileos' method (P<0.001), further validating the underestimation trends of these models.

Moreover, findings from Mohamed et al. [15], align with ours, highlighting significant discrepancies between actual birth weights and the estimates provided by both Hadlock's (P<0.001) and Vintzileos' (P<0.001) methods.

We observed strong positive correlations between actual weights and all method estimates. Lee 2 formula emerged most precise at r=0.985, closely followed by Lee 1's at r=0.984 and TVol at r=0.979. While Hadlock's and Vintzileos' correlations were slightly lower, they remained significantly reliable.

Supporting our findings, Mohamed et al. [15], compared the accuracy of seven sonographic formulae for estimating fetus weight at term, finding significant positive correlations among real birth weight & the Vintzileos and Hadlock IV formulae, emphasizing their relevance in achieving accurate birth weight estimates.

Additionally, Hassanein et al. [19], found a significant positive association among real birth weight & EFW estimated by Hadlock's method (p<0.001), corroborating the method's reliability and consistency in clinical practice.

Performance analyses revealed TVol's promising metrics of 100% sensitivity, 81-96% specificity and 0.987 AUC, highlighting effectiveness. Hadlock's achieved comparable results. Vintzileos' showed reduced reliability. Both the Lee 1 and Lee 2 Formula stood out for their accuracy, each achieving a sensitivity of 100%, a specificity of 95.8%, and AUCs of 0.987 and 0.986, respectively, highlighting their precision in fetal weight estimation.

Our findings align with those of Hassanein et al. [19], who reported a specificity of 88.1%& a sensitivity of 82.8% for the Hadlock formula, with an AUC of 87.1%, reflecting its substantial accuracy in fetal weight estimation compared to actual weights.

Consistently, Mlodawski et al. [20], compared the Lee 1 formula against the Hadlock I formula, observing that the Lee 1 formula exhibited a sensitivity of 85% and a specificity of 88%, with an AUC indicative of high efficacy in fetal weight prediction prior to delivery in term pregnancies.

Kang et al. [21], explored the efficiency of a model using three-dimensional thigh volume ultrasound to predict fetus weight, reporting an AUC of 0.923 for Tvol. The sensitivity and specificity of Tvol were 81.5% and 87.4%, respectively, validating the model's predictive value.

This study, has several limitations that merit consideration. Firstly, the small sample size. Secondly, the cross-sectional nature of the study restricts the ability to assess changes over time in the predictive accuracy of the methods tested. Finally, while the study explores several methods for estimating fetal weight, including the use of thigh circumference and fractional thigh volume, it does not consider potential inter-observer variability in these measurements, which could affect their reliability and validity.

Conclusion

Our study revealed that Lee 2 method was the closest method to actual weights, proving most reliable clinically. Significantly, thigh circumference and fractional thigh volume when added to 2d parametrs strongly correlated to actual weight, indicating reliability. Fractional thigh volume especially exhibited impressive diagnostic value with high sensitivity and specificity for our objectives.

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Table 1: Demographic and women characteristics.

Parameters		Studied women (N=60)
Maternal age (years)	Mean± SD	29.38± 7.56
	Median	30.0
	Range	18.0- 40.0
Gestational age (weeks)	Mean± SD	38.58± 1.09
	Median	38.5
	Range	37.0- 40.29
BMI (Kg/m^2)	Mean± SD	26.32±2.99
	Median	26.56
	Range	20.7 - 33.33
Parity	Multipara	37 (61.7%)
	Nullipara	23 (38.3%)
Residence	Rural	37 (61.7%)
	Urban	23 (38.3%)

SD: standard deviation,

Table 2: Comparison between actual birth weight and estimated fetal weight by different Formula.

	Studied women (N=60)					Difference	P-value
	Mean	±SD	Median	Range			
Actual birth weight (gm)	3552.60	±689.87	3567.00	2300.0	4900.0	186.22	**<0.001 (HS)**
EFW by Hadlock's method(gm)	3366.38	±590.82	3364.45	2325.0	4687.0		
Actual birth weight (grams)	3552.60	±689.87	3567.0	2300.0	4900.0	253.0	**0.003 (HS)**
EFW by Vintzileos' method(gm)	3299.60	±630.95	3310.0	2400.0	6862.0		
Actual birth weight (grams)	3552.60	±689.87	3567.0	2300.0	4900.0	140.47	**<0.001 (HS)**
EFW by Lee 1 formula (grams)	3693.07	±778.83	3820.0	2293.0	5054.0		
Actual birth weight (grams)	3552.60	±689.87	3567.0	2300.0	4900.0	43.92	**0.012 (S)**
EFW by Lee 2 method(gm)	3596.52	±754.27	3717.92	2285.48	5086.96		

P value >0.05: Not significant (NS), P value <0.05 is statistically significant (S), p<0.01 is highly significant (HS). SD: standard deviation, TC: thigh circumference

Table 3: Correlations between actual birth weight with estimated fetal weight by different Formula.

	Actual birth weight	
	r	p- value
EFW by Hadlock's method (gm)	0.967	**<0.001**
EFW by Lee 2 method (gm)	0.985	**<0.001**
EFW by Vintzileos' method (gm)	0.649	**<0.001**
EFW by Lee 1 formula (gm)	0.984	**<0.001**
Tvol	0.979	**<0.001**
Biparietal diameter (cm)	0.883	**0.659**
Head circumference (cm)	0.936	**<0.001**
Abdominal circumference (cm)	0.962	**<0.001**
Estimated TC (mm)	0.830	**<0.001**
Femur length (cm)	0.819	**<0.001**
Actual TC (mm)	0.800	**<0.001**

p≤0.05 is significant; p≤0.01 is high significant, r: Spearman correlation coefficient
EFW: Estimated fetal weight; Tvol: Fractional thigh volume, TC: thigh circumference

Table 4: Multiple linear regression analysis for factors predicting fetal birth weight

	Unstandardized Coefficients		Standardized Coefficients Beta	t	p- value
	B	Standard error			
Maternal age (years)	-2.155	1.964	-0.024	-1.097	0.278
Gestational age (weeks)	0.907	21.420	0.001	0.042	0.966
Biparietal diameter (mm)	-0.061	1.641	-0.001	-0.037	0.970
Head circumference (mm)	28.454	29.348	0.081	0.970	0.337
Abdominal circumference (mm)	-71.642	73.452	-0.249	-0.975	0.334
Estimated TC (mm)	5.612	18.227	0.013	0.308	0.760
Femur length (mm)	-622.31	348.21	-0.321	-1.787	0.080
EFW by Hadlock's method (gm)	1.798	0.962	1.540	1.870	0.068
EFWby Lee 2 method (gm)	0.285	0.135	0.244	2.108	**0.040**
EFW by Vintzileos' method (gm)	-0.031	0.030	-0.028	-1.028	0.309
Tvol	32.572	22.502	1.338	1.448	0.154
FW by Lee 1 formula (gm)	0.646	0.201	0.729	3.219	**0.002**

EFW: Estimated fetal weight; Tvol: Fractional thigh volume, TC: thigh circumference

Table 5: Accuracy of fetal thigh circumference, fractional thigh volume and estimated fetal weight by different formulas in prediction of fetal birth weight.

	Best cut off	Sensitivity	Specificity	PPV	NPV	AUC	Accuracy	P-value
Estimated TC (mm)	15	91.7%	81.2%	83%	90.7%	0.867	87%	**<0.001**
Actual TC (mm)	15.6	100%	81.2%	84.2%	100%	0.941	94%	**<0.001**
Tvol	75.4	100%	95.8%	96%	100%	0.987	98.5%	**<0.001**
EFW by Hadlock's method (gm)	2876.8	100%	93.7%	94%	100%	0.984	98%	**<0.001**
EFW by Lee 2 method (gm)	2858	100%	95.8%	96%	100%	0.986	98.5%	**<0.001**
EFW by Vintzileos' method (gm)	2880	75%	87.5%	85.7%	77.8%	0.814	81%	**<0.001**
FW by Lee 1 formula (gm)	2970	100%	95.8%	96%	100%	0987	99.5%	**<0.001**

AUC: Area Under a Curve, p value: Probability value, NPV: Negative predictive value, PPV: Positive predictive value, EFW: Estimated fetal weight; Tvol: Fractional thigh volume

*: Statistically significant at $p \leq 0.05$

Trans-Vaginal Cervical Length and Amniotic Fluid Index in Prediction of Delivery Latency Following Preterm Pre-Labor Rupture of Membranes

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Abstract

Background: After premature pre-labor rupture of membranes (PPROM), it is challenging to forecast with precision the time to delivery (latency). Predicting delivery delay may be useful in determining when to recommend hospitalization, close observation, prenatal steroid use, and magnesium sulfate for neuroprotection. In women who report with preterm pre-labor rupture of membranes (PPROM), our goal is to ascertain if transvaginal cervical length (TVCL), amniotic fluid index (AFI), or a combination of both can predict delivery latency.

Methods: 70 pregnant women presented with PPROM between the gestational age of 28 weeks and 36 weeks +6 days were enrolled. Transvaginal ultrasound measurement of cervical length and amniotic fluid index was performed within 24 hours of admission , to assess number of women reached latency period of 7 days or more and assess other maternal and fetal parameters as number of women reached 36 +6 weeks, mode of delivery, development of chorioamnionitis, gestational age at PPROM and delivery, birthweight at delivery, presence of neonatal sepsis, Apgar score at 1 and 5 minutes and need for NICU admission and indication.

Results: The best sensitivity of TVCL in prediction of delivery latency was 100 % at TVCL>3 cm in women with AFI >5 cm. On the other hand, the best specificity was 80.5% at TVCL>3 cm in women with AFI ≤5 cm.

Conclusion: When a woman presents with preterm pre-labor rupture of the membranes, it is recommended to employ either transvaginal cervical length, amniotic fluid index, or both to forecast delivery delay.

Key words: Cervical Length , Amniotic Fluid Index , Delivery Latency , PPROM.

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INTRODUCTION

When the chorioamniotic membrane bursts before 37 weeks of pregnancy or before labor begins, it is known as preterm pre-labor rupture of membranes, or PPROM. Just 2% of pregnancies are complicated by PPROM, although it is linked to 30% of preterm births.[1]

Individuals diagnosed with premature parental loss of offspring (PPROM) sometimes require prolonged hospital stays and newborn critical care.[2] The patient and the healthcare professional can become confused due to the complexity of predicting the time to delivery, also known as latency. Determining the length of the delivery delay could help establish whether specific treatments—like hospitalization, close observation, prenatal steroids when necessary, and magnesium sulfate for neuroprotection—are required.[3]

It has been demonstrated that utilizing transvaginal ultrasonography to measure cervical length (CL) in singletons and twin gestations is a useful method for predicting the likelihood of a preterm delivery with intact membranes in women who have previously experienced a preterm birth (PTB).[4]

Serial transvaginal ultrasound has been shown to be not associated with increased risk in women with PPROM, with no noticeable rise in endometritis, chorioamnionitis, or neonatal infection.5However, since transvaginal ultrasound was historically ignored in the case of ruptured membranes, it has only been studied infrequently in the management of PPROM.[6]

Both abdominal and translabial ultrasound have been shown in studies to be ineffective in accurately measuring CL. CL measured with a translabial ultrasound was not linked to the length of the delay time after PPROM. A CL of less than 2 cm has been linked to a shorter time to delivery in a few trials.[7]

A lower amniotic fluid index (AFI) in PPROM (less than 5 cm) has been associated in the past with a shorter delay and a greater rate of delivery within 7 days compared to women with a usual AFI. It is unclear, nevertheless, how these two clinical parameters would be combined or utilized alone to predict transmission delay.[8]

METHODS

From February 2022 to February 2023, the Obstetrics and Gynecology Department of the Faculty of Medicine at the Maternity Hospitals of Ain Shams University conducted this prospective observational study. Following their agreement, seventy pregnant women were enrolled in total.

Study population:

Pregnant women with PPROM who underwent TVCL measurement after admission attending Ain Shams University Maternity Hospital with the following criteria:

Inclusion criteria:

Women not in labor, age between 18 and 40 years, women with singleton gestation, women present with PPROM between the gestational age (GA) of 28 weeks and 36 weeks +6 days, women with BMI not exceed 30 kg/m2.

Exclusion criteria:

Women who have chorioamnionitis or are in active labor (characterized as having regular, effective, painful contractions that last between 30 and 70 seconds and occur 5 to 10 minutes apart and have a cervical dilatation of at least 3 cm, as confirmed by digital examination). The main clinical signs of clinical chorioamnionitis include purulent or foul-smelling amniotic fluid, fever, uterine fundal discomfort, and maternal and fetal tachycardia (>100/min and >160/min, respectively). The most significant clinical indicator of chorioamnionitis is maternal fever, women with medical disorders with pregnancy (HTN, DM, etc.), insufficient diagnostic criteria for PPROM, women with Cerclage, patients with previous cervical

operation as cervical biopsy, multifetal pregnancy, placenta Previa, cervical length measurement not performed within 24 hours of admission, women who delivered before ultrasound performed, gestational age reassigned by ultrasound at admission and did not meet inclusion criteria, women who left hospital against medical advice, women with unknown delivery outcomes, women refused to participate in the study.

The patient gave her agreement to participate in the clinical study prior to enrollment after being given a clear explanation of its purpose, scope, and potential outcomes.In the case report, the patient's initials were the only information included. The investigators stored any other documents containing the patient's name in a safe location. To make records identifiable, the scientists kept a personal patient identification list, which included patient initials matched to patient names.

The protocol and all related documents were declared for ethical and research approval by the council of the OB/GYN department at Ain Shams University prior to the start of the study and any compliance with the local regulation followed. There was no proof that ultrasound scanning had any negative effects.

Women were enrolled in the study in accordance with the inclusion and exclusion criteria following protocol clearance.A history and physical examination, which included recording pooled vaginal fluid acquired by sterile speculum inspection, were used to diagnose PPROM. The last day of a regular menstrual cycle and, if an ultrasound was available in the early stages of pregnancy, that date were used to determine the gestational age. Every woman was admitted to the hospital and given modified bed rest. TVCL was carried out utilizing the vaginal probe of the E-CUBE 6 ultrasonic imaging equipment, which has a frequency of 5 MHz, within 24 hours of admission by ALPINION MEDICAL SYSTEMS Co., Ltd., Korea.

Three readings of CL were obtained, and the median was computed once the mother's bladder was empty and the endocervical canal was fully visible for three to five minutes. Where the anterior and posterior walls of the cervix were sonographically opposed, calipers on the internal and external os were positioned. There were no distorting fibroid, polyp, or sutures present. On a few occasions, funneling was observed.

Using an E-CUBE 6 ultrasound imaging equipment from ALPINION MEDICAL SYSTEMS Co., Ltd., Kore with an abdominal probe and a frequency of 3.5 MHz, an AFI measurement was carried out. The lineanigra and a mediolateral line that passes through the umbilicus served as the uterus's vertical and horizontal axis, respectively, dividing it into four hypothetical quadrants. The vertical dimension of the deepest pocket free of fetal remains and an umbilical cord was measured. The four pockets are measured in millimeters. The AFI is the total of the four quadrant measures.

Ampicillin 2 g intravenously every 6 hours and azithromycin 500 mg twice daily for 48 hours were administered as prophylactic antibiotics. These were followed by oral amoxicillin 250 mg every 8 hours and azithromycin 500 mg twice daily for 5 days. Dextamethasone (6 mg) will be injected intramuscularly four times, separated by 12 hours.

Cardiotocography and vital signs were used to monitor the patients in order to identify any indicators of impending labor or fetal distress. Following membrane rupture, a full blood cell count and C- reactive protein titre were measured upon admission on the 48th day, and then once a week after that. The expectant mother was cared for till 36 weeks and 6 days of pregnancy.Factors such as GA at PPROM, history of preterm prelabor rupture of membranes or preterm delivery, tobacco and drug use, history of cervical procedures, visual cervical dilation at admission, presence of vaginal bleeding,

and presence or absence of funneling at the TVCL assessment were recorded along with other demographic, medical, obstetrical, sonographic, and delivery variables.

The main result was Many women experienced a seven-day or longer period of latency. The time interval (measured in days) between PPROM and the infant's delivery was called delivery delay. The secondary results were Maternal: Prolonged interval between ROMs and TVUS, quantity of women reaching 36 + 6 weeks, style of delivery (vaginal OR caesarean), occurrence of chorioamnionitis. The fetalAPGAR scores at 1 and 5 minutes, gestational age at PPROM, gestational age at delivery, birthweight at delivery, presence of neonatal sepsis, necessity for NICU hospitalization, and indication.

Statistical Analysis

Every piece of information was recorded into a database and confirmed by another, impartial party. Demographics, maternal characteristics, neonatal outcomes, and delivery delay at seven days were all subjected to statistical analysis. Fisher's exact test and the Wilcoxon rank sum test were used to find parameters that showed statistically significant differences between the two groups. A receiver operating characteristic (ROC) curve and the Youden index were used to generate cut-off values in order to examine the correlations between TVCL, AFI, and both of them during a seven-day latency period. We estimated the median delay time based on these findings, taking into account the cut-off value of each parameter. The study used sensitivity, specificity, and predictive values to investigate the potential impact of TVCL factors, AFI, or a combination of both on the likelihood of delivery within a 7-day period. We conducted comparative assessments to see whether other factors influence latency.

Results

This A total of 80 women were assessed for eligibility before enrollment in the study; 10 of them were excluded. So, this prospective observational study was conducted on 80 with pregnant women with PPROM who underwent TVCL measurement after admission.

CONSORT Flow Diagram

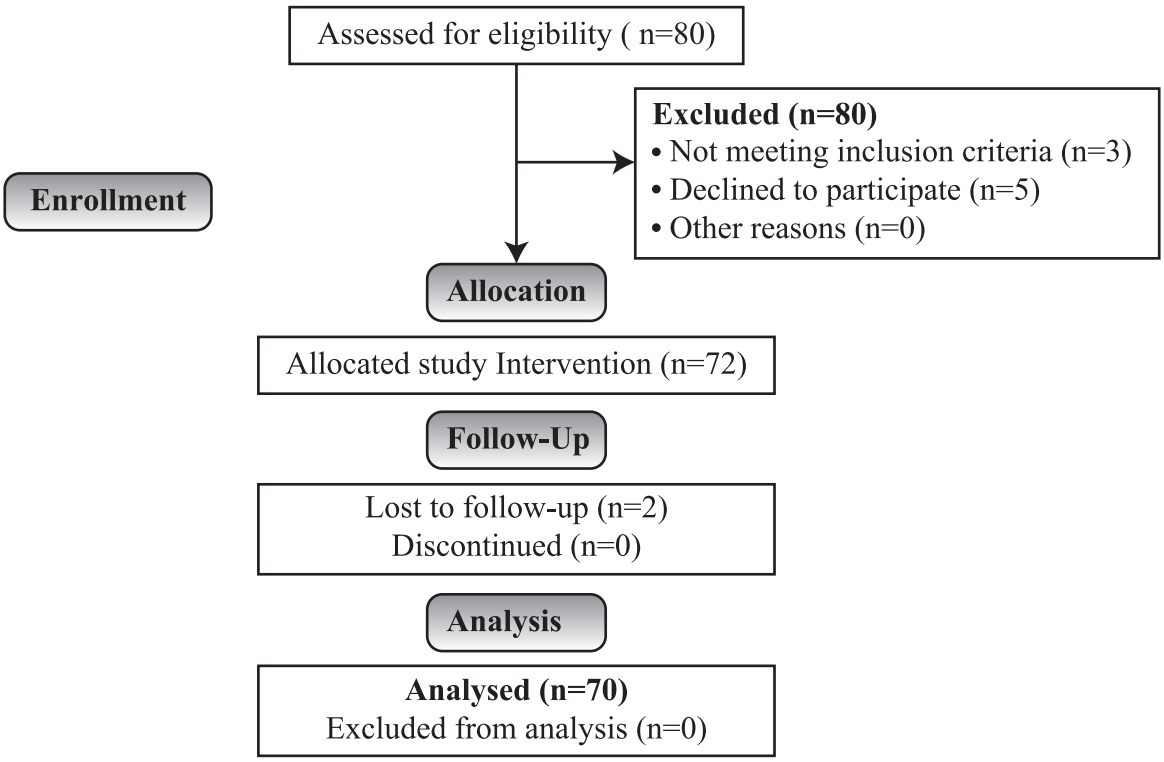


Table 1: Description of demographic data of all cases

	Mean	SD	Minimum	Maximum
AGE	27.23	6.10	18.00	39.00
BMI	27.64	1.69	22.30	30.00
GA at PPROM	33.84	2.22	28.00	36.43
			Count	**%**
Previous delivery	**CS**		39	55.7%
	NVD		8	11.4%
	no		23	32.9%
History of PPROM	**YES**		12	17.1%
	NO		58	82.9%
History of PTL	**YES**		12	17.1%
	NO		58	82.9%

Table 1 showed that mean ± SD of maternal age was 27.23 ± 6.10 yeas, BMI was 27.64 ± 1.69 kg/m2, and gestational age at PPROM was 33.84 ± 2.22 weeks. 39 cases previously delivered by CS and 8 cases by NVD. 12 cases had a history of PPROM, 12 cases had a history of PTL.

Table 2: Description of admission data of cases:

	Mean	SD	Minimum	Maximum
TVCL (mm)	27.81	7.48	11.00	50.00
AFI (cm)	4.66	2.30	1.00	10.00
CRP on Admission	**Positive**		19	27.1%
	Negative		51	72.9%

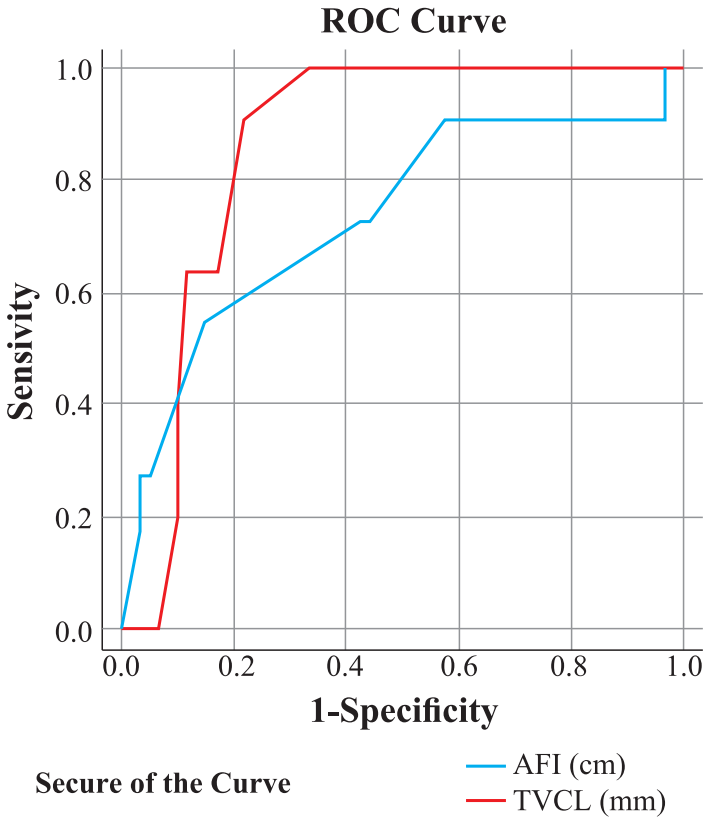
Table 2 showed that mean ± SD of TVCL was 27.81 ± 7.48 mm; AFI was 4.66 ± 2.30 cm. 19 cases had positive CRP on admission.

Table 3: Description of maternal and fetal outcomes

	Mean	SD	Minimum	Maximum
Gestational age at delivery	34.58	2.13	28.43	37.00
Birthweight at delivery (gm)	2278.51	616.04	1000.00	3750.00
APGAR score at 1 minute	6.41	1.50	1.00	9.00
APGAR score at 5 minutes	8.34	1.18	4.00	10.00
Mode of delivery	**LSCS**		45	64.3%
	NVD_INDUCED		3	4.3%
	NVD		22	31.4%
Development of chorioamnionitis	**YES**		7	10.0%
	NO		63	90.0%
NICU admission	**YES**		42	60.0%
	NO		28	40.0%
			Count	**%**
Latency period	**<=7 days**		59	84.3%
	>7 days		11	15.7%

Table 3 showed that mean ± SD of gestational age at delivery was 34.58 ± 2.13 weeks; birthweight at delivery was 2278.51 ± 616.04 gm; APGAR scores at 1 and 5 minutes were 6.41 ±1.50 and 8.34 ± 1.18 respectively.45 cases delivered by LSCS, 3 cases with induced vaginal delivery and 22 cases with NVD, 7 cases developed chorioamnionitis and 42 infants needed NICU admission, 11 cases reached > 7 days latency period.

ROC curve for prediction of Delivery Latency using TVCL (mm) and AFI (cm)



ROC curve for prediction of Delivery Latency using TVCL (mm) and AFI (cm)

	Area under the curve	P value	95% Confidence Interval		Cutt off	Sensivity %	Specificity %
			Lower Bound	Upper Bound			
TVCL (mm)	0.900	< 0.001	0.827	0.972	30.5	90.9	83.1
AFI (cm)	0.759	0.003	0.588	0.930	6.5	54.5	88.1

At cut off 30.5 to TVCL to predict delivery latency period with sensitivity and specificity 90.1%,83.1% respectively

And for AFI cut off was 6.5 and sensitivity , specificity was 54.5%,88.1% respectively.

Table 4: Accuracy of TVCL in prediction of delivery latency in all patients

	Sensitivity	Specificity	PPV	NPV
TVCL>3 cm vs ≤3 cm (n=70)	10/11=90.9%	46/59=78%	10/23=43.5%	46/47=97.9%
AFI >5 cm vs ≤5 cm (n=70)	7/11=63.6%	41/59=69.5%	7/25=28%	41/45=91.1%
TVCL>3 cm vs ≤3 cm in women with AFI >5 cm (n=25)	7/7=100%	13/18=72.2%	7/12=58.3%	13/13=100%
TVCL>3 cm vs ≤3 cm in women with AFI ≤5 cm (n=45)	3/4=75%	33/41=80.5%	3/11=27.3%	33/34=97.1%

Table 4 showed that the best sensitivity of TVCL in prediction of delivery latency was 100 % at TVCL>3 cm in women with AFI >5 cm. On the other hand, the best specificity was 80.5% at TVCL>3 cm in women with AFI ≤5 cm.

Table (5): Relation with delivery latency

		Latency period				P value
		<=7 days		>7 days		
		Count	%	Count	%	
GA at PPROM	**<=30**	5	8.5%	2	18.2%	**0.302**
	>30	54	91.5%	9	81.8%	
History of PPROM	**Yes**	11	18.6%	4	36.4%	**0.233**
	No	48	81.4%	7	63.6%	
History of PTL	**Yes**	12	20.3%	4	36.4%	**0.259**
	No	47	79.7%	7	63.6%	
Mode of delivery	**NVD_INDUCED**	3	5.1%	0	0.0%	**0.497**
	NVD	20	33.9%	2	18.2%	
	LSCS	36	61.0%	9	81.8%	
NICU admission	**Yes**	35	59.3%	6	54.5%	**1**
	No	24	40.7%	5	45.5%	
TVCL	**<=3 cm**	49	83.1%	1	9.1%	**< 0.001**
	>3 cm	10	16.9%	10	90.9%	
AFI	**<=5 cm**	42	71.2%	4	36.4%	**0.038**
	>5 cm	17	28.8%	7	63.6%	

Table 5 shows that there was significant relation between latency period and TVCL , AFI p value <0.001 and 0.038 respectively

Table (6): Latency of different parameters in prediction of delivery latency

	Latency period				P value
	<=7 days		>7 days		
	Mean	Standard Deviation	Mean	Standard Deviation	
TVCL (mm)	26.12	7.15	34.73	2.90	< 0.001
AFI (cm)	4.19	1.81	6.45	2.58	0.001

Table 6 shows that there was significant difference between latency period either < 7 or > 7 with TVCL and AFI respectively

Table (8): Multiple logistic regression models predicting delivery latency

Delivery latency	**TVCL (mm)**	0.008	1.164	1.041	1.301
	AFI (cm)	0.019	1.627	1.085	2.441

Multiple logistics regression shows significant association between TVCL ,AFI with delivery latency

DISCUSSION

According to our research, for women with an AFI more than 5 cm, TVCL had the highest sensitivity of 100% when it came to predicting delivery latency at TVCL>3 cm. However, in women with AFI ≤5 cm, the best specificity was 80.5% at TVCL>3 cm.The results of this study show that AFI by itself, at the cutoff point of 6.5 cm, has a sensitivity and specificity of 54.5% and 88.1%, respectively, in predicting delivery within 7 days.Cervical length alone at the cutoff point of 30.5 mm had a sensitivity and specificity of 90.9% and 83.1%, respectively, in predicting delivery within 7 days.In terms of forecasting delivery within 7 days, the combination of TVCL>3 cm vs. ≤3 cm had the following values: 74.29%, 56.25%, 78.79%, and 50%, respectively. The combination of AFI>5 cm vs ≤5 cm had the following predictive values: sensitivity, specificity, positive and negative predictive values for delivery within 7 days: 63.6%, 71.2%, 29.2%, and 91.3%, respectively. In women with an AFI >5 cm, the combination of TVCL>3 cm vs. ≤3 cm had 100%, 82.4%, 70%, and 100% of the sensitivity, specificity, positive, and negative predictive values for predicting delivery within 7 days. In women with AFI ≤5 cm, the combination of TVCL>3 cm vs ≤3 cm had the following combinations for predicting delivery within 7 days: 75%, 83.3%, 30%, and 97.2%, respectively. Gupta et al.9 concurred with us and said that combining AFI and TVCL increased the positive predictive value for predicting delivery delay; hence, women with AFI ≤5 and TVCL ≤2.5 cm had an 85.6% chance of giving birth within 7 days following PPROM.

Ilhan et al.10 agreed with us and reported that latent period; was positively correlated with cervical length. Cervical length was found to be the most significant factors associated with the latent period. Patients with a latent duration of 7 days or more had a statistically significantly higher cervical length than the group with an average length of >7 days (35,4 ± 8,9 ml); and patients with a latent duration between 2-7 days had higher cervical length 21,2 ± 15,0 compared to the group of 2 days or less 29.9 ± 21,8 ml.

Against our study, the study of Mehra et al.11 to predict the latent period, the combination of AFI + TVCL was evaluated, but it was not found to be significant for prediction. However, short cervical length was found to be associated with low AFI values.

In the same line with us, in Test et al.12 study, patients were retrospectively examined for 10 years, 1399 patients with PPROM were included in the study, and factors related to the latent period were discussed. Among the factors thought to shorten the latency period, oligohydramnios was found to be statistically significant.

This was in line with a study by Vermillion et al.13 that demonstrated a shorter delivery latency is linked to an AFI <5 cm after PPROM between 24 and 32 weeks of gestation.

Likewise, a recent study by Mehra et al.11 found that in women presenting with PPROM, a shorter TVCL independently predicted delivery within 7 days, while TVCL >2 cm significantly increased the likelihood of remaining undelivered at 7 days after CL evaluation.

According to Kansara and Yadav14, Patil et al.15, and Rajan and Menon16, this finding suggests that shorter latency is associated with the existence of a short cervix in PPROM.

Out of 106 women, 95 went into spontaneous labor and were included in the study, according to Kansara and Yadav14. 49 women delivered after the test, and 46 women delivered within 7 days of the test. 34 (73.91%) of the 46 women had an amniotic fluid index of less than 5 cm, while 31 (67.39) of the women had a cervical length less than 2 cm.

El Sokkary et al.17 determined how the cervical length, amniotic fluid index, and labor latency interval related to preterm premature rupture of the membranes (PPROM) relate to each other. They reported a considerable reduction in the amniotic fluid index and a shortening of the cervical length, which is consistent with our findings. Moreover, there was a direct correlation between the cervical length and the AFI and the latency interval. In comparison to controls, PPROM cases had a considerable reduction in AFI and a significant shortening of the cervical length. Cervical length and AFI were significantly correlated with the delay interval.

As regard maternal outcomes, our study reported that gestational age at delivery was 34.58 ± 2.13 weeks, 45 cases delivered by LSCS, 3 cases with induced vaginal delivery, 22 cases with NVD, 7 cases developed chorioamnionitis and 11 cases reached > 7 days latency period with no significant relation with cervical length or amniotic fluid index.

Gupta et al.9 examined the AFI and CL as predictors of pregnancy outcome in cases presenting with PPROM, measured the effectiveness of these parameters as predictors of pregnancy outcome in cases presenting with PPROM, and ascertained the AFI in cases presenting with PPROM, as well as the CL ultrasonographically. Contrary to what we said, they found a correlation between a long CL (TVCL >2.5 cm) and an AFI >5 and an increased risk of maternal morbidity, including chorioamnionitis, abruption, and cord prolapse.

In contrast, Borna et al.18and Moberg et al.19 discovered a strong link between oligohydramnios and an increased incidence of chorioamnionitis. As the length of PPROM grows, the risk of additional maternal morbidity, such as abruption (4.7 vs. 11.1%) and cord prolapse (3.1 vs. 8.3%), rose; nevertheless, the difference between the two groups was statistically non-significant.

As regard fetal outcomes, our study reported that birthweight at delivery was 2278.51 ± 616.04 gm, APGAR scores at 1 and 5 minutes were 6.41 ± 1.50 and 8.34 ± 1.18 respectively and 42 infants needed NICU admission with no significant relation with cervical length or amniotic fluid index.

Gupta et al.9 was against us and proved significant relation between cervical length or amniotic fluid index and neonatal outcomes. The majority of the newborns in Group 1 had an Apgar score of between 4 and 6, while Group 2 had a score of >6. Most newborns in both groups had an Apgar score of greater than six after five minutes. Group 1 required NICU admission at a statistically significant greater rate than Group 2. Although it was not statistically significant, Group 1 had higher rates of newborn death and morbidity.

Patil et al.15 declared that, of 170, the majority (95) belonged to the group with 28+1 to 32 weeks. The gestational period had an inverse relationship with latency ($p<0.0001$). In all three groups, a longer cervical length was associated with higher latency, a higher risk of chorioamnionitis, and more newborn problems. Additionally, compared to women with PPROM having AFI ≤5 cm, who had a shorter mean latency period (7.63±1.07 days) and a lower risk of developing chorioamnionitis, women with PPROM having AFI >5 cm had a greater mean latency period (8.32±1.25 days) and an increased risk of developing chorioamnionitis.

Lu et al.20 examined the latency period's function and possible influencing elements in order to offer guidance for the clinical management of PPROM. The incidence of low birth weight and newborn respiratory

distress syndrome (NRDS) varied significantly between the 48–168 h group and the > 168 h group with respect to neonatal outcomes (all $p < 0.05$).

CONCLUSION

The best sensitivity of TVCL in prediction of delivery latency was 100 % at TVCL>3 cm in women with AFI >5 cm. On the other hand, the best specificity was 80.5% at TVCL>3 cm in women with AFI ≤5 cm.

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Maternal and Neonatal Outcomes of Expectantly Managed Pregnancies of Healthy Cases with Previable Rupture of Membranes at Qena University Hospital

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Abstract

Background : Early premature rupture of membranes (PPROM) before foetal viability complicates obstetric treatment, putting women at risk of infection, haemorrhage, and psychological anguish and newborns at danger of respiratory distress Gestational age, cervical dilatation, nulliparity, foetal development difficulties, oligohydramnios, twin gestation, and chorioamnionitis impact PPROM delay post-viability.

Aim: To analyze maternal and neonatal outcomes in PPROM cases between 20-28 weeks of pregnancy to identify potential outcome predictors.

Methods : Qena University Hospital's retrospective observational cohort research (June 2020–June 2023) comprised singleton PPROM pregnancies between 20–28 weeks. Active labour, chorioamnionitis, foetal abnormalities, recent iatrogenic ROM, multiple gestations, and immediate delivery are excluded. Maternal demographics, obstetric history, treatments (antibiotics, glucocorticoids, magnesium sulphate), and neonatal outcomes (birth weight, Apgar scores, NICU admissions, pulmonary issues, intraventricular haemorrhage, periventricular leukomalacia, necrotizing enterocolitis, and sepsis.

Results: Of the participants (mean age 27.39 years, BMI 25.07 kg/m²), 39.02% were normal weight, 34.15% overweight, and 18.29% obese Diabetes or hypertension was present in 7.32%, PROM in 24.39%, and premature labour in 30.49%. The mean ROM gestational age was 24.93 weeks, with birth in 30.11 weeks. Caesarean delivery 48.78%, vaginal 51.22%. Non-viable pregnancies had earlier ROM and delivery ages, higher Caesarean rates, and more chorioamnionitis and maternal sepsis. Neonatal survivors had higher Apgar scores, birth weights, and pulmonary hypoplasia and sepsis rates than non-survivors.

Conclusion: Early premature deliveries make PPROM management difficult. Variations in medical procedures need customised care. NICU-admitted newborns have poor neonatal outcomes, requiring tailored care and outcomes initiatives.

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Introduction

Early premature rupture of membranes (PPROM) before foetal viability complicates obstetric care. Expectant therapy of spontaneous amniotic membrane rupture before 24 weeks gestation must be carefully considered. Obstetricians must understand maternal and newborn outcomes of expectant care for previable rupture of membranes to make educated decisions (1).

Expectant management challenges the balance between safe pregnancy progression and maternal and newborn problems. Risks for mothers include intrauterine infections such chorioamnionitis, hemorrhagic complications including placental abruption, and the psychological toll of neonatal health uncertainty. Preterm delivery, whether spontaneous or induced, complicates maternal health (2).

Neonatal outcomes in previable membrane rupture pregnancies are closely connected to extreme preterm. These babies are at risk for respiratory distress syndrome, intraventricular haemorrhage, and bronchopulmonary dysplasia. Long-term amniotic exposure increases infection risk and pulmonary hypoplasia risk, especially when membrane rupture occurs early in gestation. Understanding these outcomes is essential for providing thorough and compassionate care to mothers and their infants (3).

Current study examines factors affecting preterm premature rupture of membranes (PROM) post viability (>24 weeks) latency. Lower gestational age, higher cervical dilatation, nulliparity, foetal development limitation, and oligohydramnios are associated with shorter latency in the 24-34 week period. Latency is also shortened by twin gestation and symptomatic chorioamnionitis. However, second-trimester PROM variables are still poorly understood (4).

This study aimed to analyze maternal and neonatal outcomes in patients with previable rupture of membranes (PPROM) between 20_28 weeks of pregnancy to identify potential outcome predictors.

Patients and Methods

The project's technical design involves a retrospective observational cohort study carried out at the Obstetrics and Gynaecology Department at Qena University Hospital between June 2020 and June 2023 . This study was a proceeding for our previous study of (5). The study centered on patients who met certain criteria. The inclusion criteria consisted of patients who were treated at Qena Woman Hospital, had singleton pregnancies, experienced previable rupture of membranes (ROM), and had gestational ages between 20 and 28 weeks. The exclusion criteria for this study were as follows: the presence of active labour before or at the onset of previable rupture of membranes (ROM), signs of active chorioamnionitis upon admission, visible foetal structural anomalies detected during ultrasound examination, iatrogenic rupture of membranes within 2 weeks of amniocentesis or chorionic villus sampling, rupture of membranes occurring after viability but before the onset of labour, a latency period of less than 24 hours, and the presence of multiple foetal gestations.

The study's operational design entailed a comprehensive examination of medical records to identify pregnancies that met the criteria for eligibility. More precisely, we included women who had a single pregnancy and experienced premature rupture of membranes (PROM) during the second trimester, specifically between 20 and 28 weeks of pregnancy. To be included, these women had to have a latency period of at least 24 hours. Various diagnostic methods were used to determine if there was a rupture of membranes (ROM). These methods included visually inspecting amniotic fluid

passing from the cervical canal and pooling in the vagina using a sterile speculum examination, conducting a basic pH (positive nitrazine) test on vaginal fluid, examining dried vaginal fluid under a microscope to look for arborization (ferning), or measuring the amniotic fluid index (AFI) which should be less than 4 cm. Additionally, the patient's reported history of significant loss of vaginal fluid before 28 weeks of gestational age was taken into consideration. To maintain the quality of the study sample, certain criteria were used to exclude women. Those who showed clinical signs of chorioamnionitis upon arrival, experienced labour within 24 hours of membrane rupture, had a major foetal anomaly, or had PROM within 2 weeks of chorionic villus sampling/ amniocentesis were excluded. In order to maintain the consistency and accuracy of the study results, women who chose to have immediate delivery upon diagnosis of premature rupture of membranes (PROM) were also not included in the analysis.

The study comprehensively gathered data from the medical records of patients, including multiple aspects of maternal and obstetric care. The documented therapies for preterm premature rupture of membranes (PPROM) upon readmission were carefully recorded, which included the administration of latency antibiotics, a regimen of glucocorticoids to promote foetal lung maturity, and magnesium sulphate for foetal neuroprotection. During the time from when the patient was readmitted to when she gave birth, continuous inpatient observation was conducted to ensure thorough monitoring of the mother's health and well-being.

The maternal data collected from the records consisted of demographic variables, including age and body mass index (BMI). In addition, the researchers recorded the gravidity, which refers to the overall number of pregnancies regardless of the outcome, and the parity, which indicates the number of viable children born after 20 weeks of gestation. Additionally, any prior occurrences of premature deliveries were documented. Obstetric data yielded vital information about the timing of events, such as the gestational age when the membranes ruptured and when delivery occurred. The latency interval, which refers to the duration between the rupture of membranes and birth, was meticulously documented. The documentation was rigorous in recording information about the administration of antibiotics before delivery, the method of delivery (vaginal or caesarean section), and any difficulties that occurred throughout the pregnancy, such as chorioamnionitis, maternal sepsis, and cord prolapse. Furthermore, the duration of hospitalisation and utilisation of resources were assessed by calculating the maternal length of stay in the hospital, which includes initial observation, readmission, delivery, and postpartum inpatient care.

The study systematically gathered neonatal data, including multiple crucial elements of newborn health and outcomes. This involved recording cases of intrauterine foetal demise, which is the term used to describe the death of a foetus while still in the mother's womb after the 20th week of pregnancy. In addition, the neonatal birth weight, which was measured with a digital scale to the nearest 0.01 kg, offered important information about the growth and development path of the newborns. The Apgar ratings, measured at 1 and 5 minutes after birth, were meticulously documented. The values ranged from 0 to 10, with higher scores indicating superior overall health and adjustment to life outside the womb (6).

	Sign	Score		
		2	1	0
A	Appearance (skin colour)	Normal over entire body	Normal exept extremities	Cyanotic or pale all over
P	Pulse (heart rate)	> 100 bpm	< 100 bpm	Absent
G	Grimace response (refle xes)	Sneezes coughs, pulls away	Grimace	No response
A	Activity (muscle tons)	Active	Arms and legs flexed	Absent
R	Respiration (breathing rate and effort)	Good, crying	Slow, irregular	Absent

Furthermore, the study highlighted the necessity of being admitted to the neonatal intensive care unit (NICU), which indicates the seriousness of neonatal problems and the extent of medical attention needed. The duration of specialised medical attention was recorded, reflecting the length of stay in the NICU. The study thoroughly evaluated neonatal survival outcomes, which included three parameters: admission to the Neonatal Intensive Care Unit (NICU) with survival until discharge, admission to the NICU followed by neonatal death before discharge, or neonatal death without NICU admission.

The study carefully recorded neonatal diagnoses upon discharge from the neonatal intensive care unit (NICU), providing insight into the many health problems experienced by neonates. One of the diagnoses found was pulmonary hypoplasia, which refers to the condition of the lungs being underdeveloped or not fully grown. In addition, bronchopulmonary dysplasia was observed, which is characterised by inflammation and scarring in the lungs. This condition is commonly linked to the use of mechanical ventilation and oxygen therapy. Respiratory distress was observed, characterised by fast breathing, grunting, flaring of nostrils, and retractions of the chest wall.

In addition, intraventricular haemorrhage (IVH) was categorised into different categories, with categories III and IV being defined as severe IVH. Grade I refers to bleeding that is confined to the germinal matrix, whereas Grade II indicates intraventricular haemorrhage without enlargement of the ventricles. Grade III indicated intraventricular haemorrhage (IVH) with ventricular dilatation that filled over 50% of the ventricle, whereas Grade IV indicated IVH with bleeding within the brain tissue (7). Another diagnostic that was established is periventricular leukomalacia, which is characterised by brain damage in the white matter and the necrosis of white matter around the lateral ventricles.

Furthermore, instances of necrotizing enterocolitis, a grave illness characterised by inflammation and tissue death in the bowel, were documented. The evaluation of neonatal sepsis, caused by a proven bacterial infection, was thoroughly conducted using precise criteria (8). The criteria consisted of a body temperature above 38°C or below 36°C, a heart rate over 90 beats per minute, hyperventilation indicated by a respiratory rate above 20 breaths per minute or a PaCO2 below 32 mmHg, and a white blood cell count above 12,000 cells/μL or below 4,000 cells/μL. The diagnosis of each case was meticulously defined, guaranteeing precision and uniformity in evaluating the health outcomes of newborns.

Study outcomes

Primary outcome: The primary objective of this study was to evaluate the quality of care delivered to women undergoing inpatient

management with PROM compared with a recently instituted hospital protocol.

Secondary (subsidiary): A secondary objective was to investigate the maternal and neonatal outcomes of conservative management of Previable ROM at 20-28 weeks gestational ages in Qena University hospital, and to determine the impact of the protocol on hospital stay (bed occupancy rate).

Data analyzed using SPSS 25.0. Methods: Expressing data as number/percentage for qualitative variables and mean ± SD for quantitative ones. Statistical analysis included mean for central tendency and SD for dispersion. Comparison using t-test for two groups' means, checked against t-table for significance. Mann-Whitney test for non-normally distributed data, and Chi-square test for association between variables. Significance level set at $p < 0.05$, where smaller p values denote higher significance.

Results

Table (1): General data of included subjects

	Value (N = 82)
Age (Years)	27.39 ± 5.81
BMI (Kg/m^2)	25.07 ± 4.53
Underweight	7 (8.54%)
Normal	32 (39.02%)
Overweight	28 (34.15%)
Obese	15 (18.29%)
Gravidity	3.46 ± 2.3
Parity	2.2 ± 1.71
Abortion	0.93 ± 1.33
Medical History	
Anaemia	2 (2.44%)
Aphge	1 (1.22%)
Diabetes Mellitus	6 (7.32%)
Hypertension	6 (7.32%)
Renal	1 (1.22%)
Rheumatic Heart Disease	1 (1.22%)
Thalassemia	1 (1.22%)
History of previous PROM	20 (24.39%)
History of previous Preterm Labor	25 (30.49%)
WGA at rupture of membranes (Weeks)	24.93 ± 2.92
Latency period (Weeks)	5.11 ± 4.8
WGA at time of delivery (Weeks)	30.11 ± 6.17
Route of delivery	
CS	40 (48.78%)
NVD	42 (51.22%)

The participants had a mean age of 27.39 years (SD = 5.81), and the average Body Mass Index (BMI) was 25.07 kg/m^2 (SD = 4.53), with 8.54% underweight, 39.02% normal weight, 34.15% overweight, and 18.29% obese. Gravidity averaged 3.46 ± 2.3, parity was 2.2 ± 1.71, and the abortion rate was 0.93 ± 1.33. Medical history included conditions like anemia (2.44%), aphge (1.22%), diabetes mellitus (7.32%), hypertension (7.32%), renal issues (1.22%), rheumatic heart disease (1.22%), and thalassemia (1.22%). Additionally, 24.39% reported previous premature rupture of membranes (PROM), and 30.49% had a history of preterm labor. The mean weeks of gestational age at ROM were 24.93 ± 2.92, latency period in weeks was 5.11 ± 4.8, and weeks of gestational age at delivery were 30.11 ± 6.17. Delivery modes comprised 48.78% Caesarean sections (CS) and 51.22% normal vaginal deliveries (NVD).

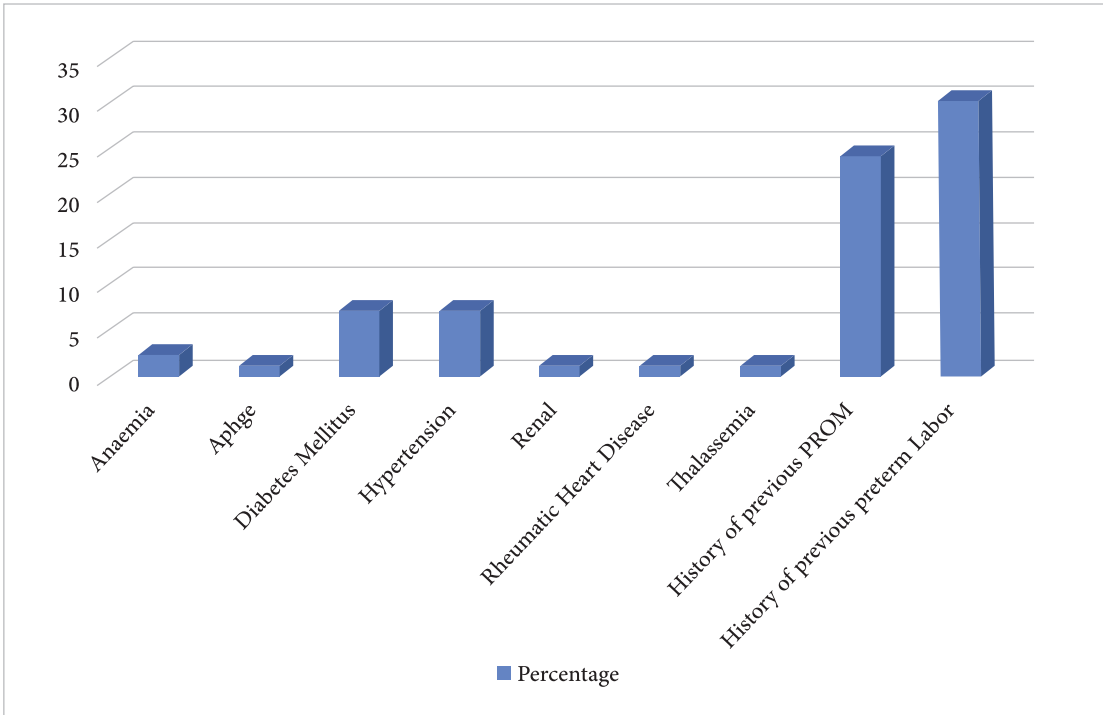

Figure (1): Medical History among included subjects

Table (2): Comparison between viable and not viable fetuses regarding maternal data

	Viable (N = 57)	Not Viable (N = 25)	P. Value
Age (Years)	27.72 ± 5.98	26.64 ± 5.33	0.4447
Gravidity	3.47 ± 2.16	3.44 ± 2.59	0.9521
Parity	2.16 ± 1.59	2.29 ± 1.96	0.781
Abortion	0.88 ± 1.22	1.05 ± 1.57	0.6286
Medical History			
Anaemia	2 (3.51%)	0 (0%)	0.3491
Aphge	1 (1.75%)	0 (0%)	0.5112
Diabetes Mellitus	5 (8.77%)	1 (4%)	0.4512
Hypertension	5 (8.77%)	1 (4%)	0.4512
Renal	0 (0%)	1 (4%)	0.1319

Rheumatic Heart Disease	1 (1.75%)	0 (0%)	0.5112
Thalassemia	1 (1.75%)	0 (0%)	0.5112
History of previous PROM	12 (21.05%)	8 (32%)	0.2937
History of previous Preterm Labor	15 (26.32%)	10 (40%)	0.2202
WGA at rupture of membranes (Weeks)	26.21 ± 2.19	22.01 ± 2.17	<0.0001*
Latency period (Weeks)	6.68 ± 4.55	1.51 ± 3.14	<0.0001*
WGA at time of delivery (Weeks)	33.2 ± 4.47	23.07 ± 2.85	<0.0001*
Route of delivery			
CS (Caesarean Section)	38 (66.67%)	2 (8%)	<0.0001*
NVD (Normal Vaginal Delivery)	19 (33.33%)	23 (92%)	
Maternal complications			
Chorioamnionitis	2 (3.51%)	10 (40%)	<0.0001*
Maternal sepsis	1 (1.75%)	3 (12%)	0.0481*
Cord prolapse	1 (1.75%)	5 (20%)	0.0031*
Antibiotic			
Type (Ultracellin or Ceftriaxone)	57 (100%)	17 (68%)	<0.0001*
Dose (1.5 g/12 h… 1g/24h)	57 (100%)	17 (68%)	<0.0001*
Route (I.V.)	57 (100%)	17 (68%)	<0.0001*
Glucocorticoids			
Dose (6mg/8 h)	42 (73.68%)	3 (12%)	<0.0001*
Type (Dexamethasone)	42 (73.68%)	3 (12%)	<0.0001*
Magnesium sulfate			
Loading dose (6gm/15-20 min)	9 (15.79%)	1 (4%)	0.1303
Maintenance dose (1gm/hour/24h)	9 (15.79%)	1 (4%)	0.1303

Non-significant changes were found in maternal age (27.72 ± 5.98 vs. 26.64 ± 5.33, p = 0.4447), gravidity (3.47 ± 2.16 vs. 3.44 ± 2.59, p = 0.9521), parity (2.16 ± 1.59 vs. 2.29 ± 1.96, p = 0.781), and abortion rates (0.88 ± 1.22 vs. 1.05 ± 1.57, Medical history showed few differences, including anemia (3.51% vs. 0%, p = 0.3491), aphge (1.75% vs. 0%, p = 0.5112), diabetes mellitus (8.77% vs. 4%, p = 0.4512), hypertension (8.77% vs. 4%), renal issues (0% vs. 4%, p = 0.1319), rheumatic heart disease (1.75% vs. 0%, p = 0.5112), and thalassemia (1.75% vs. Non-viable instances showed substantial decreases in WGA at ROM (26.21 ± 2.19 vs. 22.01 ± 2.17, p < 0.0001*), latency time (6.68 ± 4.55 vs. 1.51 ± 3.14), and WGA at delivery (33.2 ± 4.47 vs. 23.07 ± 2.85, p < 0.0001*). Significant differences were observed in Caesarean section (66.67% vs. 8%, p < 0.0001*) and normal vaginal delivery (33.33% vs. 92%) rates. Non-viable patients showed significant increases in chorioamnionitis (3.51% vs. 40%, p < 0.0001*), maternal sepsis (1.75% vs. 12%, p = 0.0481*), and cord prolapse (1.75% vs. 20%, p = 0.0031*). Significant increases in antibiotic and glucocorticoid use were observed in viable instances (100% vs. 68%, p < 0.0001*), but magnesium sulfate administration revealed no significant difference (15.79% vs. 4%, p = 0.1303).

Table (3): Comparison between viable and not viable fetuses regarding maternal Amniotic Fluid evaluation at time of admission by US

	Viable (N = 57)	Not Viable (N = 25)	P. Value
Average Amniotic Fluid	16 (28.07%)	1 (4%)	0.013*
Mild Oligohydramnios	29 (50.88%)	3 (12%)	0.0007*
Severe oligohydramnios	2 (3.51%)	3 (12%)	0.1425
Anhydramnios	9 (15.79%)	18 (72%)	<0.0001*
Polyhydramnios	1 (1.75%)	0 (0%)	0.5112
Placenta Previa	2 (3.51%)	0 (0%)	0.3491
Placental Separation	1 (1.75%)	1 (4%)	0.5497
Polycystic Kidneys	1 (1.75%)	0 (0%)	0.5112
Fibroid	1 (1.75%)	0 (0%)	0.5112
IUGR	1 (1.75%)	0 (0%)	0.5112
Dead Fetus	0 (0%)	1 (4%)	0.1319

Statistically significant distinctions were observed in average amniotic fluid volume (28.07% vs. 4%, p = 0.013*), mild oligohydramnios prevalence (50.88% vs. 12%, p = 0.0007*), anhydramnios incidence (15.79% vs. 72%, p < 0.0001*), and various other parameters. Minimal disparities were noted in severe oligohydramnios, polyhydramnios, placenta previa, placental separation, polycystic kidneys, fibroid, intrauterine growth restriction (IUGR), and the occurrence of a dead fetus. From all viable fetuses, 35 were admitted to the NICU and 22 weren't admitted to the NICU.

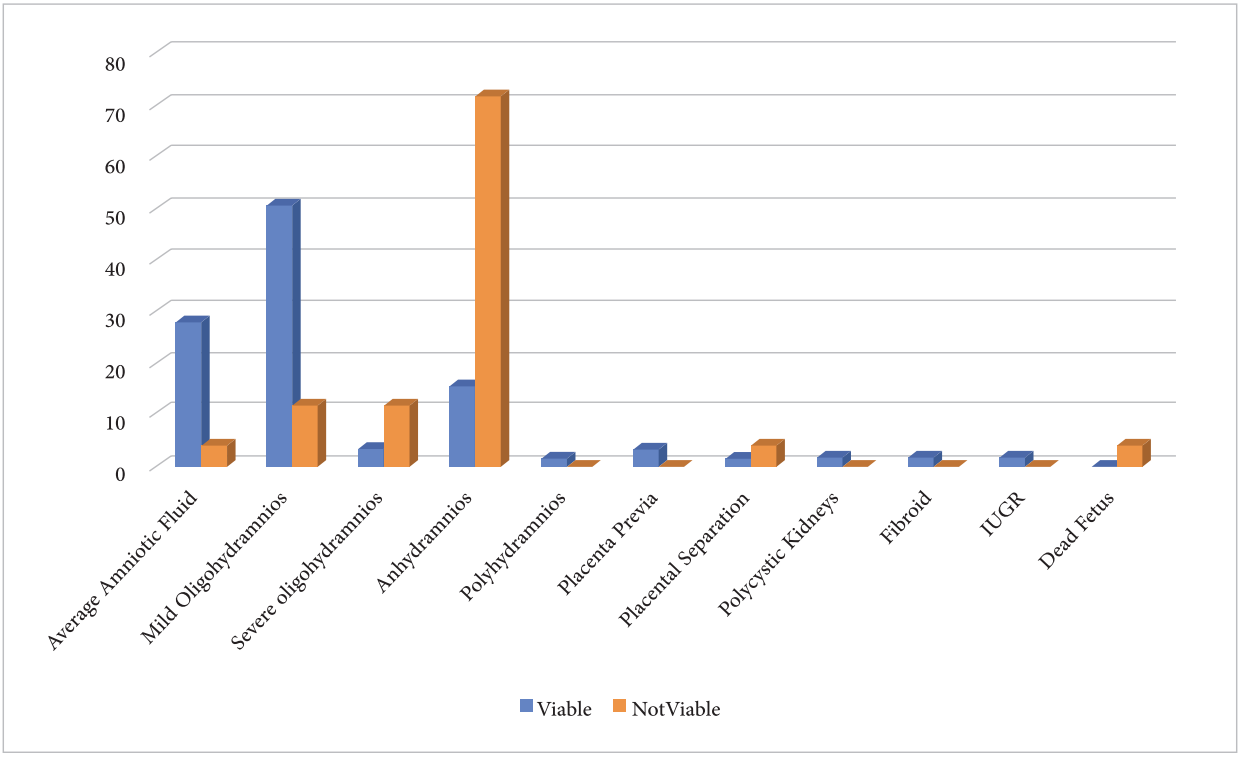

Figure (2): Comparison between viable and not viable fetuses regarding maternal Amniotic Fluid evaluation at time of admission by US

Table (4): Comparison between NICU survival neonates and those who died at NICU regarding maternal data

	NICU Survival (N = 20)	Death at NICU (N = 15)	P. Value
Age (Years)	27.9 ± 6.37	28.27 ± 4.84	0.8576
Gravidity	3.3 ± 2.03	3.93 ± 1.69	0.3479
Parity	2.12 ± 1.28	2 ± 1.31	0.809
Abortion	0.76 ± 0.88	1.29 ± 1.67	0.2895
Medical History			
Anaemia	0 (0%)	1 (6.67%)	0.2541
Aphge	1 (5%)	0 (0%)	0.3945
Diabetes Mellitus	1 (5%)	1 (6.67%)	0.8394
Hypertension	2 (10%)	1 (6.67%)	0.7367
Renal	0 (0%)	0 (0%)	
Rheumatic Heart Disease	0 (0%)	1 (6.67%)	0.2541
Thalassemia	0 (0%)	0 (0%)	
History of previous PROM	2 (10%)	7 (46.67%)	0.0131*
History of previous Preterm Labor	9 (45%)	3 (20%)	0.1305
WGA at rupture of membranes (Weeks)	26.64 ± 1.83	25.97 ± 2.01	0.3239
Latency period (Weeks)	6.74 ± 3.23	2.63 ± 3.21	0.001*
WGA at time of delivery (Weeks)	33.65 ± 2.8	28.69 ± 3.43	0.0001*
CS	16 (80%)	9 (60%)	0.206
NVD	4 (20%)	6 (40%)	
Maternal complications			
Chorioamnionitis	0 (0%)	2 (13.33%)	0.086
Maternal sepsis	0 (0%)	1 (6.67%)	0.2541
Cord prolapse	0 (0%)	1 (6.67%)	0.2541
Antibiotic			
Type (Ultracellin or Ceftriaxone)	20 (100%)	15 (100%)	-
Dose (1.5 g/12 h… 1g/24h)	20 (100%)	15 (100%)	-
Route (I.V.)	20 (100%)	15 (100%)	-
Glucocorticoids			
Dose (6mg/8h)	17 (85%)	10 (66.67%)	0.2125
Type (Dexamethasone)	17 (85%)	10 (66.67%)	0.2125
Magnesium sulfate			
Loading dose (6gm/15-20 min)	5 (25%)	2 (13.33%)	0.4081
Maintenance dose (1gm/hour/24h)	5 (25%)	2 (13.33%)	0.4081

In the comparison between neonates who survived in the Neonatal Intensive Care Unit (NICU) and those who died, maternal general data showed no significant differences, including maternal age (27.9 ± 6.37 vs. 28.27 ± 4.84, p = 0.8576), gravidity (3.3 ± 2.03 vs. 3.93 ± 1.69, p = 0.3479), parity (2.12 ± 1.28 vs. 2 ± 1.31, p = 0.809), and abortion rates (0.76 ± 0.88 vs. 1.29 ± 1.67, p = 0.2895). Similarly, medical history parameters showed no significant differences. However, focusing on maternal gestational age (GA) metrics at rupture of membranes (ROM) and delivery, neonates who survived in the NICU demonstrated a significant increase in the latency period (6.74 ± 3.23 vs. 2.63 ± 3.21, p = 0.001*) and weeks of gestational age (WGA) at delivery (33.65 ± 2.8 vs. 28.69 ± 3.43, p = 0.0001*). Caesarean section rates (80% vs. 60%) and normal vaginal delivery rates (20% vs. 40%) did not exhibit significant differences. Maternal complications, including chorioamnionitis, maternal sepsis, and cord prolapse, also displayed no significant differences between the two groups. Examination of maternal medications during pregnancy revealed no significant differences in antibiotic administration (100% vs. 100%) and glucocorticoid use (85% vs. 66.67%).

Table (5): Comparison between NICU survival neonates and those who died at NICU regarding maternal Amniotic Fluid evaluation at time of hospital admission by US

	NICU Survival (N = 20)	Death at NICU (N = 15)	P. Value
Average Amniotic Fluid	2 (10%)	0 (0%)	0.2188
Mild Oligohydramnios	13 (65%)	9 (60%)	0.7702
Severe oligohydramnios	1 (5%)	1 (6.67%)	0.8394
Anhydramnios	4 (20%)	4 (26.67%)	0.6537
Polyhydramnios	0 (0%)	1 (6.67%)	0.2541
Placenta Previa	1 (5%)	1 (6.67%)	0.8394
Placental Separation	0 (0%)	1 (6.67%)	0.2541
Polycystic Kidneys	0 (0%)	1 (6.67%)	0.2541
Fibroid	1 (5%)	0 (0%)	0.3945
IUGR	0 (0%)	1 (6.67%)	0.2541

Assessing amniotic fluid evaluation at hospital admission via ultrasound, NICU survival neonates exhibited no significant differences compared to those who died. No notable disparities were observed in average amniotic fluid volume, oligohydramnios, severe oligohydramnios, anhydramnios, polyhydramnios, placenta previa, placental separation, polycystic kidneys, fibroid, and intrauterine growth restriction (IUGR).

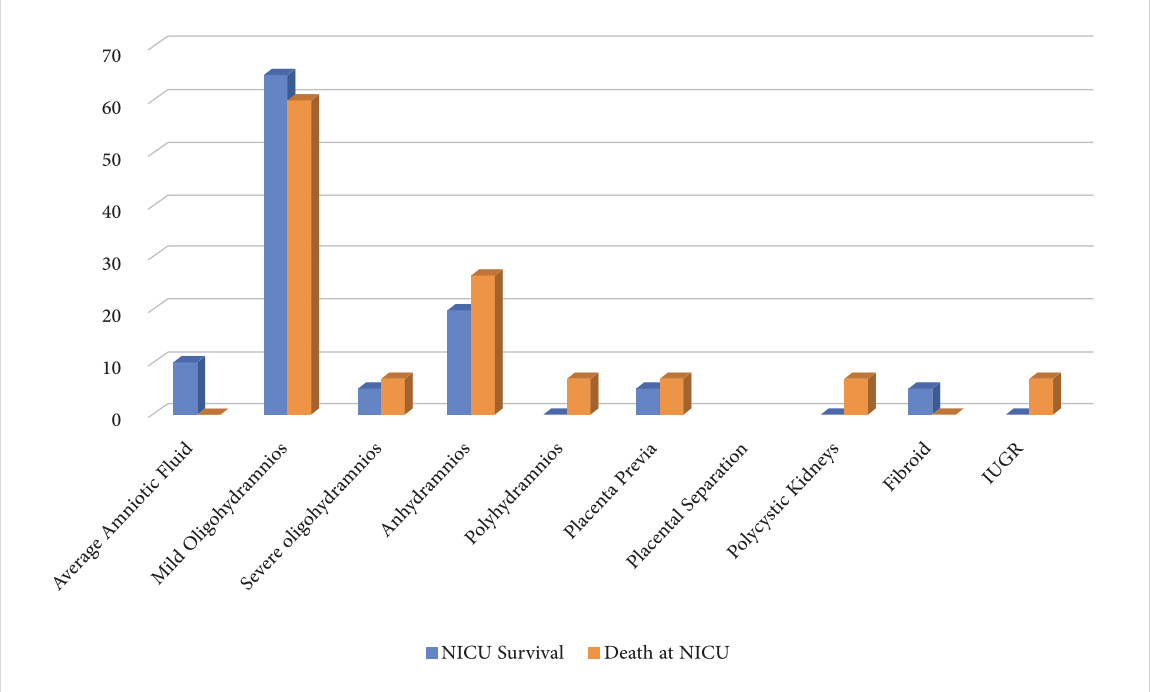

Figure (3): Comparison between NICU survival neonates and those who died at NICU regarding maternal Amniotic Fluid evaluation at time of hospital admission by US

Table (6): Comparison between NICU survival neonates and those who died at NICU regarding fetal outcomes

	NICU Survival (N = 20)	Death at NICU (N = 15)	P. Value
Neonatal outcomes			
Apgar score			
1 minute	6.4 ± 1.2	4.4 ± 1.31	0.0001*
5 minutes	7.05 ± 1.53	4.67 ± 1.62	0.0001*
Neonatal birth weight	2.52 ± 0.36	1.69 ± 0.63	<0.0001*
Length of stay (days)	9.81 ± 6.61	6.15 ± 4.7	0.1038
Neonatal complications			
Pulmonary hypoplasia	11 (55%)	14 (93.33%)	0.012*
Respiratory Distress Syndrome	8 (40%)	10 (66.67%)	0.1254
Neonatal sepsis	4 (20%)	9 (60%)	0.0145*
Intraventricular hemorrhage	0 (0%)	1 (6.67%)	0.2541
Pneumonia	4 (20%)	3 (20%)	0.99

In terms of neonatal outcomes, significant differences were observed in Apgar scores at 1 minute (6.4 ± 1.2 vs. 4.4 ± 1.31, p = 0.0001*) and 5 minutes (7.05 ± 1.53 vs. 4.67 ± 1.62, p = 0.0001*), with the survival group showing higher scores, while neonatal birth weight exhibited a significant decrease (2.52 ± 0.36 vs. 1.69 ± 0.63, p < 0.0001*). All neonates were admitted to the NICU in both groups, with no significant difference in length of stay (9.81 ± 6.61 vs. 6.15 ± 4.7, p = 0.1038). Regarding neonatal complications, there was a significant increase in the incidence of pulmonary hypoplasia among those who died compared to the survival group (55% vs. 93.33%, p = 0.012*). Respiratory distress syndrome showed a non-significant increase in the

death group (40% vs. 66.67%, p = 0.1254), while neonatal sepsis exhibited a significant increase in the death group (20% vs. 60%, p = 0.0145*). Intraventricular hemorrhage and pneumonia showed no significant differences between the two groups (0% vs. 6.67%, p = 0.2541 and 20% vs. 20%, p = 0.99, respectively).

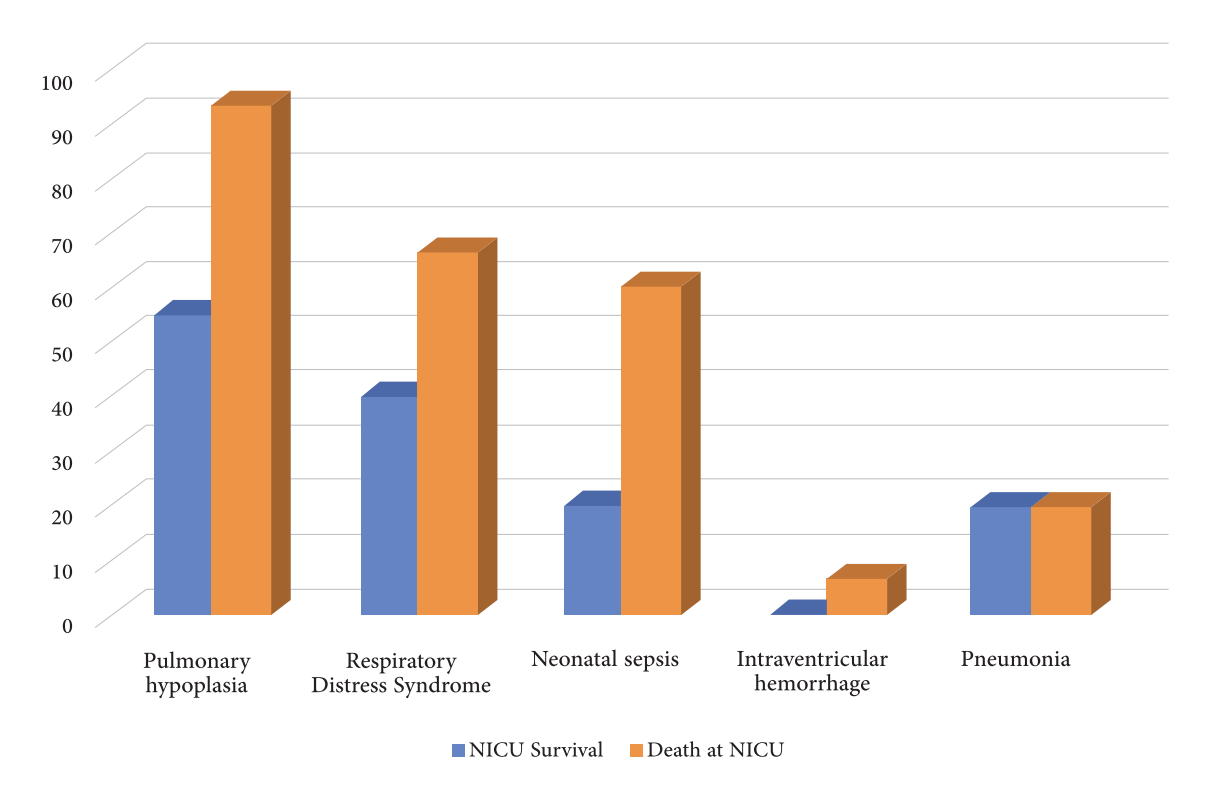

Figure (4): Comparison between NICU survival neonates and those who died at NICU regarding Neonatal complications.

Discussion

Our study reported a mean mother age of 27.39, gravidity of 3.46, parity of 2.2, and abortion rate of 0.93. Preterm labor (30.49%) and early rupture of membranes (24.39%) are notable. Mother overall data was the same for viable and non-viable fetuses. We found no significant differences in maternal age (28 vs. 30 years), gravida (2 vs. 2), or parity (1 vs. 1) between pre-viable and viable PPROM patients, supporting (9).

According to (10), there were no significant differences in maternal age, primigravida status, preterm labor history, or PPROM history between early and late PPROM groups (p=0.090, p=0.487, p=0.542, p=0.523, These factors were similar in early and late PPROM groups.

PPROM gestational age considerations illuminated our study. The mean gestational age at rupture of membranes (ROM) was 24.93 weeks, with a 5.11-week delay and 30.11-week delivery. Nonviable fetuses had considerably lower gestational age at ROM, latency length, and delivery.

In agreement with our findings, (11) found a mean gestational age of 20.45 ± 2.9 weeks and a mean latency duration of 44.7 ± 34.8 days at PPROM Timing is crucial, as (12) discovered significant differences in gestational age at PPROM between early and late groups.

(13) discovered significant differences in gestational age at PPROM between expectant management and termination of pregnancy groups, underlining the necessity for age-specific therapy.

Our study used a comprehensive approach to treat preterm premature membrane rupture. Most (90.24%) received Ultracellin or Ceftriaxone to avoid infections. Clinical guidelines indicate complex therapy, so Dexamethasone (54.88%) and magnesium sulfate (12.2%) were given during fetal lung maturation in viable instances.

Fetal viability affects antibiotic and glucocorticoid therapy. Increasing antibiotic use (100% vs. 68%, $p < 0.0001$*) in viable patients emphasizes the importance of infection prevention for better outcomes. Variations in glucocorticoid use (73.68% vs. 12%, $p < 0.0001$*) emphasize the necessity for viability-based therapy.

(14) advised proactive antibiotic treatment to minimize intrauterine infections. For better maternal outcomes, (15) recommended diligent antibiotic monitoring.

Our study used 54.88% glucocorticoids, compared to 37.8% in (16). Different managerial practices are suggested.

Mild oligohydramnios (39.02%) and anhydramnios (32.93%) dominated amniotic fluid. Viable and non-viable pregnancies differed in amniotic fluid volume (28.07% vs. 4%, $p = 0.013$*), mild oligohydramnios prevalence (50.88% vs. 12%, $p = 0.0007$*), and anhydramnios incidence (15.79% vs. 72%, p

(17) found 88.2% perinatal mortality from anhydramnios. After PPROM, (18) linked oligohydramnios to lower Apgar scores and longer NICU stays. Another study linked oligohydramnios severity to neonatal survival.

(9) discovered no link between amniotic fluid volume and oligohydramnios severity and neonatal outcomes. Study gestational age ranges may explain this variation. Our study focuses on previable ROM (20-28 weeks), but ÖZEL et al.'s study includes PPROM (12-33 weeks), potentially altering results.

51.22% were normal vaginal deliveries and 48.78% Caesarean sections. The study demonstrated that viability status impacts delivery outcomes, with viable cases having a higher NVD rate (33.33% vs. 92%, $p < 0.0001$*) and non-viable cases having a higher CS rate (66.67% vs. 8%, $p < 0.000$*

Our CS rate (48.78%) exceeds (17) 27.6% in singleton PPROM pregnancies before 24 weeks. (9) discovered significant CS rate differences between pre-viable and viable PPROM groups (27.5% vs. 65.2%, $p < 0.001$).

In expectantly managed preterm premature rupture of membranes (pPPROM), (2) identified a 21.1% CS rate and delivery style impacting newborn mortality. Our maternal issues included chorioamnionitis (14.63%), maternal sepsis (4.88%), and cord prolapse (7.32%), unlike Mung-Yuen and Tsz-Kin (2017) and Linehan and Walsh (2020

(19) discovered 64.7% medical terminations, 19.6% spontaneous abortions, and 29.4% intraamniotic infections. (12) report reduced severe maternal morbidity and mortality. Prenatal prognosis is challenging despite therapy advancements.

Newborns had a 69.51% viability rate, with Apgar scores of 6.6 ± 2.28 at 1 minute and 7.04 ± 2.31 at 5 minutes. NICU admissions at 61.4% and fetal survival at 73.68%. Chorioamnionitis, sepsis, and nonviable cord prolapse were maternal problems.

Subgroup analysis of viable NICU admissions found variations in our study. Out of 35 NICU-admitted neonates, 57.14% (20) survived and 42.86% (15) perished. NICU-admitted neonates showed lower latency period, WGA, mild oligohydramnios, and lower amniotic fluid volume. NICU survivors also had longer latency and gestational ages. Our study found no significant differences in maternal medication habits, delivery procedures, or Apgar ratings, but deceased newborns had higher rates of pulmonary hypoplasia and neonatal infection.

(14) documented newborn consequences include pulmonary hypoplasia (29.5%), congenital infection (56.8%), and intraventricular hemorrhage (25%). (20) noted that earlier gestational age at PPROM negatively affected newborn prognosis. (19) observed 28.6% infant mortality due to pulmonary hypoplasia and diverse morbidity. For surviving infants, (21) reported significant incidences of respiratory distress syndrome, neonatal sepsis, bronchopulmonary dysplasia, and intraventricular hemorrhage

Neonatal survival rates vary despite advancements. (15) observed NICU-admitted newborns had a 95% fatality rate, while (22) found 18.7% and 42.8% survival rates for distinct gestational age groups. (17) found a 73.3% perinatal mortality rate, with most survivors having good neurodevelopment but respiratory issues.

Conclusion

In conclusion, our study on expectantly managed pregnancies with previable rupture of membranes reveals challenges in early preterm births. Variations in medical interventions highlight the need for tailored care. Adverse neonatal outcomes in NICU-admitted neonates emphasize the necessity for targeted strategies in this vulnerable population, aiding clinicians and researchers in enhancing care and outcomes.

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Incidence of ovarian hyperstimulation Syndrome among patients with polycystic ovarian syndrome undergoing IVF/ICSI

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Abstract

Background: IVF/ICSI is the preferred treatment option for PCOS-related infertility, however, the risk of ovarian hyperstimulation syndrome (OHSS) is significantly increased.

Aim: Therefore, this study was conducted to estimate incidence and degree of OHSS among PCOS women underwent IVF/ICSI after taking different stimulation protocol in Mansoura fertility unit.

Methods: The study enrolled 108 PCOS cases underwent IVF/ICSI. OHSS occurrence was detected and possible risk factors for moderate to severe OHSS were studied.

Results: Results revealed that the group of (Antagonist protocol with a GnRH Agonist trigger) had 2.2% OHSS incidence. The group of (Antagonist protocol plus hCG trigger) had an OHSS incidence of 22.6%. The group of (Long Agonist protocol plus hCG trigger) had an OHSS incidence of 25%. The choice of IVF/ICSI protocol (e.g. pituitary suppression protocol, oocyte maturation trigger & freeze all vs fresh embryo transfer) in addition to multiple demographic (e.g. Age & BMI), historical (e.g. LOD), clinical (e.g. AFC), laboratory (e.g. AMH) and follow up (e.g. E2 level at trigger day, Number of follicles >18mm, Number of oocytes retrieved, Number of transferred embryos, Clinical pregnancy, multiple pregnancy) factors affected OHSS incidence significantly.

Conclusion: To reduce the likelihood of Ovarian Hyperstimulation Syndrome (OHSS) occurring, certain measures can be taken, including employing the GnRH antagonist protocol for inhibiting the pituitary gland and Stimulating ovulation through the use of a GnRH agonist, as well as cryopreservation of all embryos (IVF/ICSI cycle segmentation). Close monitoring of PCOS patients during IVF/ICSI with treatment plans individualization.

Keywords: Polycystic Ovary Syndrome (PCOS), In Vitro Fertilization/Intracytoplasmic Sperm Injection (IVF/ICSI), Ovarian Hyperstimulation Syndrome (OHSS), Human Chorionic Gonadotropin (hCG), Laparoscopic Ovarian Drilling (LOD), Estradiol (E2), Gonadotropin-Releasing Hormone (GnRH), Anti-Mullerian Hormone (AMH), Antral Follicle Count (AFC), Body Mass Index (BMI)..

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is an ovarian stimulation complication used for yielding numerous ovarian follicles simultaneously which is an important step during assisted reproductive techniques. OHSS is a rare side effect that has different degrees of severity including mild, moderate up to severe & even lethal (1). The most important dangers are linked to moderate & severe OHSS resulting in thromboembolic accidents, acute renal impairment & respiratory distress requiring assisted ventilation (2, 3).

The rate of incidence of OHSS in PCOS patients undergoing IVF/ICSI significantly increased up to 13.9 times more than the incidence in non PCOS patients diagnosis (4). The definite etiology of OHSS is not known. The management is empirical and so prophylaxis is the top management line of OHSS (5).

Recognizing risk factors and predictive factors for OHSS and individualizing the controlled ovarian stimulation (COS) protocol in a good manner is the most important step in the primary prophylaxis of OHSS, because each individual has his own response to each COS strategy as regard to risks and benefits (6).

Patients and Methodology

This retrospective research was carried out in Mansoura Fertility Unit and Mansoura university hospitals during the period from January 2016 to March 2020. The study included 108 infertile PCOS patients diagnosed by criteria of (7) who were aged 18 to 40 years of age and have undergone IVF/ICSI procedures. But we excluded Patients who were infertile due to factors other than PCOS e.g. severe male factor (azoospermia), severe endocrinal disorders (severe thyroid dysfunction and/or severe hyperprolactinemia) and anatomical causes of infertility e.g. Uterine hypoplasia, bicornuate uterus and unicornuate uterus.

Consideration of ethics

Research protocol was submitted and received approval from the Medical Research Ethical Committee at Faculty of Medicine, Mansoura University (code: MS.20.02.1027). It was submitted and officially approved by the board of obstetrics and gynecology departments, Mansoura University.

Methods

Data from the cycles of IVF/ICSI of PCOS patients were collected from the fertility unit records. Data on OHSS outcomes were obtained from the medical records of the patients in the obstetrics and gynecology departments of Mansoura University hospitals. Collected patients' data included:

- Base line demographic data e.g. age, BMI, Infertility type, Infertility duration, Basic investigations e.g. semen analysis, HSG, hormonal assay (e.g. FSH, LH, TSH, Prolactin, AMH) and AFC by US in day 2 of cycle.
- Past medical and surgical history whether +ve or -ve.
- Data related to OHSS e.g. past hx of OHSS, grade, type, E2 level at trigger day, Lab. Investigations (e.g. WBCs count, Hematocrit, serum albumin level, SGOT, SGPT, serum creatinine level, platelet count), ascites whether +ve or -ve, US findings (e.g. dominant follicle size and number of follicles >18mm), hospital admission.
- Data related to oocytes and embryo grading e.g. count of oocytes collected, count of embryos, freeze all or not, fresh embryo transfer or not, number of transferred embryos of grade A(D3).
- Pregnancy data e.g. chemical pregnancy, clinical pregnancy, ongoing clinical pregnancy (viable), multiple pregnancy.

- Data of symptoms, signs and management modalities of OHSS according to its grade e.g. thromboembolic events, ascites, pleural effusion, coagulation abnormalities, multiple system failure, renal shut down, paracentesis, LMWH administration, intensive critical care and albumin administration.

This data was processed statistically to determine OHSS incidence, degree and risk factors.

Outcomes

Primary outcomes

- Incidence of OHSS: "proportion of patients who develop Ovarian Hyperstimulation Syndrome within a specific group." It is typically expressed as a percentage. This statistic helps assess the risk of OHSS associated with different treatment approaches.

Secondary outcomes

- OHSS grade is a classification system used to categorize the severity of Ovarian Hyperstimulation Syndrome (OHSS). This system typically categorizes OHSS into different grades based on specific criteria, such as symptoms, physical exam findings, and ultrasound scans. Each grade reflects the increasing intensity of OHSS presentation. It's used to assess the risk associated with Various protocols for ovarian stimulation.
- **BMI and OHSS:** The correlation between the body mass index of the patient (BMI) and the likelihood of them developing OHSS.
- **Age and OHSS:** These describe how a patient's age can be a factor in developing OHSS.
- **Clinical pregnancy and OHSS:** correlation between pregnancy and OHSS incidence.
- **AFC by US on day 2 of cycle and OHSS incidence:** This explains how a particular ultrasound metric, the count of antral follicles (AFC), can be employed to evaluate the risk of OHSS. AFC quantifies the count of tiny follicles in the ovaries on the second day of a menstrual cycle.
- **AMH and OHSS incidence:** The correlation between AMH and OHSS refers to the **statistical association** between the level of anti-Müllerian hormone (AMH) in a woman and her **possibility of experiencing ovarian hyperstimulation syndrome (OHSS) during** fertility treatment.
- **Laparoscopic ovarian drilling and OHSS: The relationship between laparoscopic of ovarian drilling (LOD) and OHSS** refers to the potential impact of a surgical procedure called **laparoscopic ovarian drilling** on the possibility of experiencing ovarian hyperstimulation syndrome (OHSS) during fertility treatment.

Estradiol level at trigger day and OHSS: The relationship between estradiol level at trigger day and OHSS refers to the potential **association** between the concentration of the hormone **estradiol** assessed on the day of ovulation trigger **(trigger day)** and the potential for developing **ovarian hyperstimulation syndrome (OHSS)** during fertility treatment.

Analysis of Statistical Data

We entered and processed data using IBM-SPSS software Version 26.0. Qualitative data was represented as N (%), and quantitative data was denoted as mean, standard deviation (SD). Quantitative data was initially examined for normality using Shapiro-Wilk's test and Kolmogorov-Smirnov's test, with data deemed normally distributed if $p>0.05$. The existence of significant outliers (extreme values) was verified by inspecting boxplots. For qualitative data across groups, the Chi-Square (χ^2) test was employed if the expected count in all cells was ≥ 5 (adequate

sample size), otherwise Fisher's exact test was utilized. The Independent-Samples t-test was applied to compare quantitative data that follows a normal distribution between two groups. One way ANOVA (One way Analysis Of Variance) was used to compare normally distributed quantitative data among more than two groups using the F-test. The Mann-Whitney U test was employed for non-parametric data. A P value less than or equal to 0.05 was considered to be significant.

Results

The group following the Antagonist protocol in addition to a GnRH Agonist trigger had an OHSS incidence of 2.2%. The group following the Antagonist protocol along with an hCG trigger had an OHSS incidence of 22.6%. The group following the Long Agonist protocol along with an hCG trigger had an OHSS incidence of 25%. Due to limitations in the chi-square test for contingency tables with small sample sizes ($n < 5$), particularly evident in the observed incidence of OHSS in the first group, Fisher's exact test was used for P-value calculation. To ensure precise estimation of P-values with this test, a modified 2x2 contingency analysis was conducted. There was a significant difference in OHSS incidence between the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Antagonist protocol with an hCG trigger (p1), as well as between the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Long Agonist protocol with an hCG trigger (p3). However, no significant difference detected between the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger (p2). (Table 1).

Table (1) Incidence of Ovarian Hyperstimulation Syndrome (OHSS) among groups of different IVF/ICSI protocols according to both pituitary suppression protocol & oocyte maturation trigger.

		(Ant.+Agonist trig.) group (45 patients)	(Ant.+HCG) group (31 patients)	(Long Ag.+HCG) group (32 patients)	P1	P2	P3
Incidence of OHSS N(%)	No	44 (97.8%)	24 (77.4%)	24 (75%)	0.005*	0.8	**0.002***
	Yes	1 (2.2%)	7 (22.6%)	8 (25%)			

*A P-value is considered significant if it is ≤ 0.05. The percentages displayed in the table are within-group percentages. The 1st Group followed the Antagonist protocol with a GnRH Agonist trigger, the 2nd Group followed the Antagonist protocol with hCG trigger, and the 3rd Group followed the Long Agonist protocol with hCG trigger. P1 represents the P-value for the difference between the 1st and 2nd groups, P2 represents the P-value for the difference between the 2nd and 3rd groups, and P3 represents the P-value for the difference between the 1st and 3rd groups.

A non-significant difference in baseline characteristics, including age, BMI, type/duration of infertility, antral follicle count (AFC), and hormonal assays, were observed among patients undergoing different IVF/ICSI protocols. To enable a statistical comparison of semen analysis and hysterosalpingography results among patients undergoing various IVF/ICSI protocols, a 2x2 contingency analysis was employed. This method was chosen considering the limited sample size, as it ensures a more precise calculation of the P-value (statistical

significance) using Fisher's exact test. According to the baseline demographic data, no statistically significant variation ($p > 0.05$) was observed between either semen analysis or hysterosalpingography results and the different patient groups in the present study. A review of the patients' medical history, including laparoscopic ovarian drilling showed no differences among the various patient groups. (Table 2)

Table (2): Base line of demographic data.

		(Ant.+Agonist trig.) group (45 patients)	(Ant.+HCG) group (31 patients)	(Long Ag.+HCG) group (32 patients)	P value		
Age (Year) (Mean ±SD).		29.49 ± 4.526	28.39 ± 3.6	28.53 ± 4.79	0.48		
BMI (kg/m²) (Mean ±SD).		29.8 ± 3.74	29.6 ± 5.44	28.4 ± 4.9	0.406		
Infertility type N (%)	1ry	39 (86.7%)	21 (67.7%)	26 (81.3%)	0.127		
	2ry	6 (13.3%)	10 (32.3%)	6 (18.8%)			
Infertility duration (year) (Mean ±SD).		5.8 ± 2.5	4.97 ± 2.36	4.9 ± 2.038	0.2		
Hormonal assay: (Mean ±SD).	FSH	4.84 ± 2.02	4.65 ± 1.89	8.38 ± 14.69	0.065		
	LH	8.1 ± 5.53	9.999 ± 4.96	9.04 ± 5.1	0.15		
	TSH	2.5 ± 1.9	2.18 ± 1.07	2.31 ± 1.38	0.815		
	Prolactin	13.4 ± 5.66	13.5 ± 4.6	13.7 ± 4.67	0.97		
	AMH	4.93 ± 3.56	5.36 ± 3.07	5.41 ± 3.88	0.728		
AFC by US in Day 2 of cycle (Mean ±SD).		32.8 ± 2.29	33.7 ± 2.7	34.03 ± 2.5	0.088		
Basic investigations: Semen Analysis N (%)	Normal	38 (84.4%)	30 (96.8%)	28 (87.5%)	P1 0.09	P2 0.2	P3 0.7
	Moderately affected	7 (15.6%)	1 (3.2%)	4 (12.5%)			
HSG N (%)	Normal	41 (91.1%)	29 (93.5%)	29 (90.6%)	P1 0.7	P2 0.7	P3 0.9
	Abnormal	4 (8.9%)	2 (6.5%)	3 (9.4%)			

Gyneco-logical surgery N (%)	LOD	+ve	13 (28.9%)	5 (16.1%)	3 (9.4%)	P1 0.6	P2 0.5	P3 0.2
		-ve	32 (71.1%)	26 (83.9%)	29 (90.6%)			
	Operative laparoscopy	+ve	13 (28.9%)	5 (16.1%)	3 (9.4%)	P1 0.6	P2 0.5	P3 0.2
		-ve	32 (71.1%)	26 (83.9%)	29 (90.6%)			

A P-value is deemed significant if it is ≤ 0.05. The percentages shown in the table represent within-group percentages. P1 denotes the P-value for the difference between the group that followed the Antagonist protocol with a GnRH Agonist trigger and the group that followed the Antagonist protocol with an hCG trigger. P2 denotes the P-value for the difference between the group that followed the Antagonist protocol with an hCG trigger and the group that followed the Long Agonist protocol with an hCG trigger. P3 denotes the P-value for the difference between the group that followed the Antagonist protocol with a GnRH Agonist trigger and the group that followed the Long Agonist protocol with an hCG trigger.

Our analysis revealed significant differences in estradiol (E2) levels on the trigger day among patients undergoing different IVF/ICSI protocols. Additionally, a complete blood count (CBC) analysis, including white blood cell (WBC) count, platelet count, and hematocrit, showed variations depending on the protocol. Similarly, serum glutamic oxaloacetic transaminase (SGOT) and creatinine levels, along with platelet count, exhibited protocol-dependent differences.

Various IVF/ICSI protocols showed no significant difference between groups in serum albumin, SGPT levels, the number of follicles >18mm (by US), or dominant follicle size (by US). To calculate a more accurate P-value using Fisher's exact test, a 2x2 data analysis was performed. The need for hospital admission and the presence of ascites (fluid buildup in the abdomen) were more common in the (Antagonist protocol with hCG trigger) and (Long agonist protocol with hCG trigger) groups, which exhibited statistically significant differences in both when compared with the (Antagonist protocol with GnRH agonist trigger) group (P1 and P3). However, there was no statistically significant difference between the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger in terms of hospital admission and ascites. (Table 3)

Table (3): Detailed OHSS data.

		(Ant.+Agonist trig.) group (45 patients)	(Ant.+HCG) group (31 patients)	(Long Ag.+HCG) group (32 patients)	P value		
Past history of OHSS N (%)	+ve	1 (2.2%)	0	0	P1 0.4	P2 -	P3 0.4
	-ve	44 (97.8%)	31 (100%)	32 (100%)			

OHSS grade	Moderate N (%)	1 (2.2%)	3 (9.68%)	3 (9.38%)	P1 0.3	P2 0.8	P30.3
	Severe N (%)	0	4 (12.9%)	5 (15.63%)			
Type of OHSS	Early N (%)	1 (2.2%)	3 (9.68%)	4 (12.5%)	P1 0.3	P2 0.8	P3 0.4
	Late N (%)	0	4 (12.9%)	4 (12.5%)			
Estradiol (E2) level at trigger day (Mean ±SD).		1966 ± 2405.8	2990 ± 2889.7	3069.5 ± 1393.8	0.001*		
Laboratory Investigations (Mean ±SD).	WBCs/ mm3	7679.8 ± 2797.6	12197 ± 7186.33	11231.5 ± 8089.9	0.003*		
	Hematocrit%	35.5 ± 4.5	41.6 ± 8.29	40.85 ± 8.99	0.001*		
	Serum albumin level mg/dl	4.3 ± 0.5	3.8 ± 1.37	3.6 ± 1.5	0.688		
	SGOT U/ml	22.5 ± 10.6	30.3 ± 15.5	31.6 ± 20.86	0.042*		
	SGPT U/ml	25.2 ± 5.4	41.4 ± 36.4	40.85 ± 36.35	0.331		
	Serum creatinine level mg/dl	0.86 ± 0.17	1.07 ± 0.35	1.07 ± 0.38	0.01*		
	Platelet count K/uL	286.6 ± 84.4	335.4 ± 95.7	334.15 ± 102.57	0.03*		
Ascites N (%)	Positive (+)	1 (2.2%)	7 (22.6%)	8 (25%)	P1 0.005*	P2 0.8	P3 0.002*
	Negative (-)	44 (97.8%)	24 (77.4%)	24 (75%)			
Ultrasound (Mean ±SD).	Number of follicles >18mm	11.93 ± 6.24	14.94 ± 7.52	14.22 ± 4.14	0.08		
	Dominant follicle size	19.51 ± 1.79	19.84 ± 2.07	19.88 ± 1.43	0.56		
Hospital Admission N (%)		1 (2.2%)	7 (22.6%)	8 (25%)	P1 0.005*	P2 0.8	P3 0.002*

*A P-value is deemed significant if it is ≤ 0.05. The percentages shown in the table represent within-group percentages. P1 denotes the P-value for the difference between the group that followed the Antagonist protocol with a GnRH Agonist trigger and the group that followed the Antagonist protocol with an hCG trigger. P2 denotes the P-value for the difference between the group that followed the Antagonist protocol with an hCG trigger and the group that followed the Long Agonist protocol with an hCG trigger. P3 denotes the P-value for the difference between the group that followed the Antagonist protocol with a GnRH Agonist trigger and the group that followed the Long Agonist protocol with an hCG trigger.

The number of oocytes retrieved significantly differed among patients undergoing different IVF/ICSI protocols. However, there was no statistically significant association between the IVF/ICSI protocol employed and the resulting number of embryos. Owing to the limited size of the sample, a Fisher's exact test, which requires a smaller sample size, was used. The type of pituitary suppression protocol and oocyte maturation triggers significantly impacted the choice between freezing all embryos and fresh transfer within different patient groups. This is likely due to varying OHSS risks and the effect of the trigger on endometrial receptivity. There was a difference that was statistically significant. when the group following the Antagonist protocol with a GnRH Agonist trigger was compared with either the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger regarding the number of transferred embryos of grade A (D3). (Table 4)

Table (4): Oocytes and embryo grading.

		(Ant.+Agonist trig.) group (45 patients)	(Ant.+HCG) group (31 patients)	(Long Ag.+HCG) group (32 patients)	P value		
The average number of oocytes collected (Mean ±SD).		19.2 ± 7.5	24.23 ± 10	31.7 ± 11.6	0.001*		
Number of embryos (Mean ±SD).		10.73 ± 5.29	11.19 ± 4.32	11.75 ± 4.18	0.73		
Freeze all N (%)	Yes	45 (100%)	8 (25.8%)	2 (6.3%)	P1 0.001*	P2 0.04*	P3 0.001*
	No	0	23 (74.2%)	30 (93.8%)			
Fresh embryo transfer N (%)	Yes	0	23 (74.2%)	31 (96.9%)	P1 0.001*	P2 0.011*	P3 0.001*
	No	45 (100%)	8 (25.8%)	1 (3.1%)			
Number of transferred embryos of grade A (D3) N (%)	0	45 (100%)	8 (25.8%)	2 (6.3%)	P1 0.001*	P2 0.9	P3 0.001*
	1	0	9 (29%)	15 (46.9%)			
	2	0	14 (45.2%)	14 (43.8%)			
	3	0	0	1 (3.1%)			

*A P-value is considered significant if it is ≤ 0.05. The percentages displayed in the table are within-group percentages. P1 represents the P-value for the difference between the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Antagonist protocol with an hCG trigger, P2 represents the P-value for the difference between the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger, and P3 represents the P-value for the difference between the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Long Agonist

protocol with an hCG trigger. The P-value of the "number of transferred embryos" was calculated using a 2x2 analysis comparing two groups: one with zero or one embryo and the other with more than one embryo.

There wasn't significant statistical link between the type of pituitary suppression protocol, whether antagonist or agonist, and the pregnancy rates following IVF/ICSI procedures. (Table 5)

Table (5): Pregnancy data.

N(%)		(Ant.+HCG) group (31 patients) Antagonist protocol	(Long Ag.+HCG) group (32 patients) Agonist protocol	P value by Fisher's exact test
Chemical pregnancy	Yes	8 (25.8%)	4 (12.5%)	0.182
	No	23 (74.2%)	28 (87.5%)	
Clinical pregnancy	Yes	8 (25.8%)	4 (12.5%)	0.182
	No	23 (74.2%)	28 (87.5%)	
Ongoing clinical pregnancy (Viable)	Yes	8 (25.8%)	4 (12.5%)	0.182
	No	23 (74.2%)	28 (87.5%)	
Multiple pregnancy	Yes	2 (6.5%)	3 (9.4%)	0.67
	No	29 (93.5%)	29 (90.6%)	

The group following the Antagonist protocol with a GnRH Agonist trigger was excluded from the analysis of pregnancy data because all patients in this group underwent freezing of all embryos. A P-value is considered significant if it is ≤ 0.05. The percentages displayed in the table are within-group percentages.

The analysis of OHSS patients revealed a distinct difference between moderate and severe cases. Notably, all severe cases presented with ascites and pleural effusion, while only one case experienced blood clot issues. The management approach significantly differed; all severe cases required paracentesis, anticoagulation, and intensive care, compared to the moderate group where these interventions were used in a limited manner. (Table 6)

Table (6): incidence of symptoms, signs and management modalities of OHSS according to its grade.

N (%)	Moderate OHSS	Severe OHSS	
Thromboembolic events	(7 patients)	(9 patients)	P value
Ascites	0	1 (11.1%)	0.56
Pleural effusion	7 (100%)	9 (100%)	-
Coagulation abnormalities	0	9 (100%)	0.01*
Multiple system failure	0	1 (11.1%)	0.56
Renal shut down	0	0	-
Paracentesis	0	0	-
LMW heparin	1(14.3%)	9 (100%)	0.001*
Intensive critical care	7 (100%)	9 (100%)	-
Albumin administration	0	9 (100%)	0.01*
	7 (100%)	9 (100%)	-

The table presents the percentage of patients in each grade of OHSS. A P-value is considered significant if it is ≤ 0.05.

The potential risk factors for Ovarian Hyperstimulation Syndrome (OHSS) was investigated by comparing baseline characteristics, ovarian reserve markers, stimulation parameters, oocyte retrieval data, and cycle outcomes between patients who developed OHSS and those who did not. Baseline characteristics included age, body mass index (BMI), and duration of infertility. The ovarian reserve markers assessed were the antral follicle count (AFC) by ultrasound on cycle day 2 and the Anti-Müllerian Hormone (AMH) level. The history of laparoscopic ovarian drilling (LOD) was also taken into account. The stimulation parameter analyzed was the estradiol (E2) level on the trigger day for oocyte retrieval. Data on number of follicles exceeding 18mm, the number of oocytes collected, and cycle outcomes such as freeze-all cycles, fresh embryo transfers, rates of clinical pregnancy, multiple pregnancy rates, and the number of transferred embryos of high quality (grade A, Day 3) were also compared. All these factors showed significant differences between patients who experienced OHSS and those who didn't. (Table 7)

Table (7): Factors affecting OHSS incidence significantly.

Age (Year) (Mean ±SD).		26.13 ± 2.09	29.37 ± 4.47	0.005*
BMI (kg/m²) (Mean ±SD).		23 ± 1.5	30.4 ± 4	0.001*
Infertility duration (years) (Mean ±SD).		3.44 ± 0.73	5.63 ± 2.4	0.001*
AFC by US in Day 2 of cycle (Mean ±SD).		35.75 ± 2.4	33 ± 2.3	0.001*
AMH (Mean ±SD).		11.05 ± 2.7	4.18 ± 2.48	0.001*
LOD N (%).		0	21 (22.8%)	0.034*
Estradiol (E2) level at trigger day (Mean ±SD).		6760 ± 3646	1861 ± 868.8	0.001*
Number of follicles >18mm (Mean ±SD).		20.44 ± 6.15	12.26 ± 5.4	0.001*
Number of oocytes retrieved (Mean ±SD).		44.25 ± 9.7	20.89 ± 6.5	0.001*
Freeze all N (%).		4 (25%)	51 (55.4%)	0.025*
Fresh embryo transfer N (%).		12 (75%)	42 (45.7%)	0.03*
Clinical pregnancy (+ve) N (%).		8 (50%)	4 (4.3%)	0.001*
Multiple pregnancy N (%).		5 (31.3%)	0	0.001*
Number of transferred embryos of grade A (D3) N (%).	Zero or one embryo transferred	8 (50%)	71 (77.2%)	0.024*
	More than one embryo transferred	8 (50%)	21 (22.8%)	

*A P-value is considered significant if it is ≤ 0.05. The percentage displayed in the table pertains to OHSS.

Discussion

This retrospective study investigated the impact of ovarian stimulation protocols and oocyte maturation triggers on the incidence and intensity of OHSS in 108 PCOS patients who underwent IVF/ICSI. The antagonist protocol with a GnRH agonist trigger had the lowest incidence of OHSS (2.2%, moderate only) with zero incidence of severe or lethal grades, while the long agonist protocol with an hCG trigger had the highest (25%, including both moderate and severe grades). There was a significant difference between the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Long Agonist protocol with an hCG trigger. Also, the group following the Antagonist protocol with a GnRH Agonist trigger and the group following the Antagonist protocol with an hCG trigger showed significant differences, despite having the same pituitary suppression protocol (antagonist). The highest incidence was in the group triggered by hCG. There was no significant disparity between the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger despite the difference in pituitary suppression protocol (antagonist vs long agonist), but both were triggered by hCG. This suggests that the Antagonist protocol is a powerful preventive measure against OHSS in PCOS patients if oocyte maturation is triggered by a GnRH agonist, segmentation (freeze all) is practiced, and hCG is avoided. These findings align with those found in the literature by (6, 8-11).

The GnRH antagonist protocol is emerging as the preferred approach for PCOS patients due to its potential to reduce the risk of OHSS, its financial viability, and its shorter duration of stimulation, all without negatively affecting the likelihood of pregnancy outcomes (12). The present study supports this, demonstrating a lower incidence of OHSS with the antagonist protocol, especially when oocyte maturation was achieved by GnRH agonist instead of hCG. This aligns with practices aimed at removing the possibility of severe OHSS (13). Overall, the GnRH antagonist protocol is advised for patients with a high likelihood of OHSS, and substituting hCG with a GnRH agonist can further decrease the risk of severe OHSS (14).

The current study discovered that using hCG as an oocyte maturation trigger increased the likelihood of OHSS in PCOS patients undergoing IVF/ICSI. This finding aligns with the literature, which reports an association between hCG and an increased risk of OHSS (15, 16).

The current study found no difference of significance in age and BMI among patients undergoing different IVF/ICSI protocols, suggesting that age and BMI might not be a primary factor in the selection of the protocol. However, a difference that was significant in age and BMI was observed among patients who developed OHSS and those who did not. A young age, specifically ≤ 28 years, increased the incidence of OHSS. This aligns with existing literature, where a younger age is identified as a contributing factor for OHSS, regardless of the specific IVF/ICSI protocol used (17, 18). The presence of OHSS revealed a potential association with an ideal BMI. While this study and others (19) observed a trend towards a lower BMI in patients with OHSS, the existing literature is not entirely consistent. (17) identified a low BMI as a risk factor for OHSS, while (20) reported no such correlation. Further study is required to clarify the connection between BMI and the risk of OHSS.

AMH emerged as a risk factor for OHSS, with levels equal to or exceeding 8.5 ng/mL associated with a higher incidence. This aligns with existing research suggesting that extremely high AMH levels (>50 pmol/L)

increase the risk of OHSS in PCOS patients in comparison to lower AMH levels (21). AMH appears to be a more reliable Indicator of ovarian response and OHSS than either age or BMI, allowing for tailored strategies to minimize the risk of overstimulation in patients with elevated AMH (22, 23).

While the Antral Follicle Count (AFC) assessed via ultrasound on the second day of a cycle didn't significantly vary between patients who underwent different IVF/ICSI protocols, it became a risk factor for OHSS when it reached or exceeded 35. This aligns with the observation that patients who experienced OHSS tended to have a higher AFC compared to those who didn't. Studies have suggested different thresholds for predicting OHSS using AFC and other markers: (24) proposed 19.5 for AFC, 22.5 pmol/L for AMH, and 9.5 for the number of collected eggs. On the other hand, (19) reported that an AFC of 24 or higher was found to be a risk factor for moderate-to-severe ovarian hyperstimulation syndrome (OHSS) in patients with polycystic ovary syndrome (PCOS). These findings suggest that AFC can help predict OHSS. Therefore, knowing a patient's AFC levels is crucial for planning and managing the risks of OHSS during fertility treatments.

Laparoscopic ovarian drilling (LOD) seemed to be protective against OHSS, particularly in patients with PCOS. Studies like (25) suggested that a history of LOD significantly diminishes the risk of OHSS in this population. Compared to traditional gonadotropin therapy for ovulation induction, LOD offers similar results but avoids the side effects of OHSS (26).

Estradiol levels, measured on the day of ovulation trigger, significantly differed among patients undergoing various IVF/ICSI protocols. Protocols associated with a higher incidence of OHSS resulted in demonstrably elevated E2 levels within those groups. This aligns with findings from (18, 27, 28), who all reported a strong correlation between high E2 levels and OHSS development. Our study identified a high risk of OHSS in PCOS patients with E2 levels of ≥ 3500 pg/mL on the trigger day, suggesting a potential risk threshold. However, it is important to consider variations across studies. For instance, (29) proposed a cut-off of ≥5000 pg/mL, while (18) used a broader range of 3000-5000 pg/mL on the trigger day. Additionally, (27) identified E2 levels exceeding 126 ng/mL on Day 3 of the cycle and a significant fold increase by Day 10 as potential risk factors. These findings collectively suggest that E2 level can be a valuable predictor of OHSS. However, the specific cut-off value for high risk may vary depending on the study population and the specific IVF/ICSI protocol used.

In our study, it was found that having ≥ 15 follicles with a diameter exceeding 18 mm was linked to a heightened risk of OHSS in PCOS cases. On the other hand, the number of follicles > 18mm and the dominant follicle size didn't show any difference of significance between the IVF/ICSI protocol groups. Measuring follicles before retrieval can predict OHSS risk, with ≥13 follicles ≥11 mm in diameter (30). Throughout ovulation stimulation, a notably greater quantity of follicles was observed in the patient group experiencing OHSS (31).

In the present study, having ≥ 28 oocytes retrieved was recognized as a factor of risk for OHSS in PCOS cases. The study also revealed a difference with significance between groups of IVF/ICSI protocols. (27) found that AFC and the count of eggs retrieved were indicative of OHSS, with women who developed OHSS yielding a higher number of eggs per cycle. Additionally, the ideal number of oocytes retrieved is 24, and it is recommended to freeze all embryos if 25 or more oocytes are retrieved to avoid late onset OHSS (32).

Clinical pregnancy, when it occurred in PCOS cases undergoing IVF/ICSI, increased OHSS risk. Pregnancy itself is a known risk

contributor to OHSS, particularly late-onset OHSS, which typically develops 10-17 days post-treatment (22, 33). This is because rising hCG levels from pregnancy can exacerbate existing early OHSS or trigger late-onset OHSS (18). To mitigate this risk, the freeze-all approach, in which embryos are frozen and transferred in a separate cycle, can be considered to decouple pregnancy from the initial ovarian stimulation phase (34).

Freezing all embryos (the freeze-all strategy) as opposed to transferring fresh embryos appeared to be a valuable tool to lower the risk of OHSS in PCOS patients undergoing IVF/ICSI. Our investigation revealed a statistically significant relationship between the choice of oocyte maturation trigger (hCG versus GnRH agonist) and the preferred embryo transfer strategy within the antagonist protocol. Notably, patients triggered with the GnRH agonist exclusively underwent a freeze-all approach, while those triggered with hCG demonstrated a greater preference towards fresh embryo transfer. Studies have shown that compared to fresh embryo transfer, the freeze-all approach significantly reduces the risk of OHSS development while maintaining elevated rates of live births in following frozen embryo transfer cycles (35, 36). This benefit is particularly important for patients at high risk of OHSS, and the freeze-all strategy can be safely carried out using a GnRH agonist trigger (36). Furthermore, some studies suggest the freeze-all approach might even improve pregnancy rates beyond just reducing OHSS risk (35). Therefore, considering a freeze-all technique, especially when fresh embryo transfer carries a high OHSS risk, presents a valuable alternative (37).

The present study has shown that multiple pregnancies have a connection with an increased risk of OHSS, particularly the more severe form. For instance, twin pregnancies exhibit a higher tendency towards severe OHSS compared to mild or moderate forms, and the incidence of late-onset OHSS is more than double that of early-onset OHSS (38, 39).

The present study revealed a statistically significant difference in the number of transferred high-quality (Grade A, D3) embryos among patient groups with different oocyte maturation triggers. Patients triggered with the GnRH agonist exclusively underwent a freeze-all strategy, indicating no transfers of these embryos. Conversely, the hCG-triggered groups (the group following the Antagonist protocol with an hCG trigger and the group following the Long Agonist protocol with an hCG trigger) exhibited a distribution of > 50% receiving either one embryo or undergoing a freeze-all, while the remaining < 50% received more than one embryo transfer. The analysis identified the number of transferred embryos of grade A (D3) if > 1 as a risk factor for OHSS. Individuals with a higher number of transferred embryos exhibited a greater likelihood of experiencing severe OHSS (40).

Conclusion

In conclusion, the cases described highlight the potential risks associated with OHSS in patients with PCOS who are undergoing treatment with IVF/ICSI. To reduce the risk of OHSS, certain measures can be taken, including the use of antagonist protocol or GnRH agonist trigger for ovulation, as well as cryopreservation of all embryos. It is important for healthcare providers to closely monitor patients with PCOS during IVF/ICSI and individualize treatment plans to lower the risk of OHSS incidence and associated complications.

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Surgical Site Infection Following Episiotomy Repair in Relation to Routine Use of Postpartum Antibiotic Prophylaxis in Low Risk Population: A Randomized Controlled Trial

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Abstract

Background: Antibiotic prophylaxis may lower the incidence of wound infections following an episiotomy, especially in circumstances like midline episiotomy, incision extension, or environments with a high baseline risk of infection following vaginal delivery, which are linked to a higher risk of postpartum perineal infection. Nevertheless, there is conflicting data at this time about the benefit of prophylactic antibiotics in avoiding infections after an episiotomy.

Objective: Evaluation of whether regular prophylactic antibiotic medication to women after an uncomplicated vaginal delivery, as opposed to not administering any antibiotic prophylaxis, lowers postpartum maternal infectious morbidities and improves outcomes.

Methods: A total of 200 pregnant women with who underwent elective episiotomy repair were enrolled and divided into two equal groups; study group received oral antibiotics in a dose of 625gm twice daily for 3 days after delivery and control group didn't receive postpartum antibiotics. We followed up her through a telephone call weekly for 6 weeks asking about fever, discharge, vaginal pain, dysuria, vulval swelling, redness and pelvic pain. Maternal readmission to hospital, puerperal sepsis, urinary tract infection, endometritis, serious infectious complications was compared between study groups.

Results: No differences were noted between study groups regarding all study parameters. Routine antibiotics after episiotomy had no role in prophylaxis against wound complications, maternal fever, puerperal infection and maternal readmission.

Conclusion: Administration of prophylactic systemic antibiotic post episiotomy is not effective to prevent wound infection.

Keywords: Surgical Site Infection; Episiotomy; Postpartum Antibiotic.

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Introduction

In Approximately 200 000 of the projected 300 000 women who died in 2017 were in subSaharan Africa, illustrating the unacceptable global rate of maternal mortality [1].

Sepsis caused by maternal infection is a major factor in these fatalities. However, there are little data about the prevalence and etiology of maternal infection [2].

Women who are supposed to have simple vaginal deliveries may be more susceptible to bacterial infections if they have a number of pre-existing medical issues. These include illnesses such group B streptococcus infections, bacterial vaginosis, anemia, and malnourishment [3].

Furthermore, the risk of infection in the puerperium may be raised by complications related to labor and delivery (such as prolonged rupture of the membranes, prolonged labor, genital tract lacerations, and retained products of conception) or by interventions by the provider (such as frequent vaginal examinations, operative vaginal birth (using forceps or a vacuum), and episiotomy) [4].

An episiotomy is a planned incision made on the perineum during the second stage of labor, which is considered when there are signs that if it is not performed, there might be a substantial rupture of the perineum [5].

A spontaneous vaginal delivery, or SVD, is when a woman gives birth to her child via the birth canal (the vagina) without the need for forceps, vacuum extraction, or a cesarean section. This may happen when the mother is not using any medications or other methods to induce labor. Approximately four million vaginal births occur in the United States annually, with the majority being spontaneous [6].

One of the key variables influencing medical outcomes is socioeconomic status (SES). Poor SES has been linked to insufficient medical treatment and unfavorable results. Low SES may raise a woman's chance of unfavorable pregnancy outcomes [7].

Infection-prone obstetrical operations, including as caesarean sections, manual placenta removal, and the repair of third- or fourth-degree perineal injuries, should be avoided by antibiotic prophylaxis. Anatomically speaking, episiotomies resemble a second-degree perineal laceration, involving [5].

the connective tissue, underlying muscles, and vaginal mucosa, and may not justify the regular use of preventive antibiotics [8].

Prophylactic antibiotic usage for episiotomies seems to vary much, nevertheless. As far as we are aware, there is no research on the use of prophylactic antibiotics for episiotomies in 3 high-income countries, and clinical guidelines do not suggest using them in the absence of infection. However, prophylactic antibiotic use appears to be very common in certain lowincome countries, where most women undergo episiotomies and receive them [9].

The risk of episiotomy infection is reduced by general infection control practices such hand hygiene, aseptic surgical methods, site cleaning, and sterilization of tools used in the procedure [10].

Methods

From September 2022 to May 2023, a randomized controlled clinical study was carried out at the Obstetrics and Gynecology Department of the Faculty of Medicine, Ain Shams University Hospitals. The following conditions were met by expectant mothers who visited the labor ward:

Patients who had an uneventful vaginal delivery, had elective episiotomy repair, and showed no signs of continuing antibiotic use throughout the postpartum phase met the inclusion criteria.

Patients with prolonged rupture of membranes (PROM >24 hours), chorioamnionitis, prolonged second stage of labor (>2 hours), third- or fourth-degree vaginal tears, retained or manual placenta removal, post-partum

hemorrhage, anemia, diabetes, and immunocompromised status were among the exclusion criteria.

Sampling Method

Women who met the eligibility requirements were randomly allocated to either group using systematic random selection. Two hundred opaque envelopes were serially numbered, and the matching letter representing the assigned group was placed inside each envelope in accordance with the randomization table. Next, each envelope was sealed and placed within a single box. Using MedCalc ® version 13, a computergenerated randomization sheet was used for the randomization process.

Sample size justification

A sample size of 100 women per group was required to detect a difference between the two groups, using the Pass 11 software to calculate the sample size. Power was set at 80%, a-error at 0.05, and the incidence of infection in the control group was assumed to be 10% and in the intervention group to be 2%.

Ethical considerations

Before being recruited in the research, the patient's information and informed permission were obtained. The patient gave her assent to participate after being given a clear explanation of the purpose, scope, and potential drawbacks of the clinical trial. In the case report, the patient's initials were the only information included. The investigators stored any additional documents containing the patient's identity in a safe location. To make records identifiable, the scientists kept a personal patient identification list, which included patient initials matched to matching patient names. The protocol and all related paperwork were declared for ethical and research approval by the council of the OB/GYN department at Ain Shams University before the study started, and any compliance with local regulations was followed. There is no proof that the research intervention is detrimental.

Study procedures

The patients underwent a thorough history taking of clinical value, a general examination with a focus on the "Leopold maneuvers" of the obstetric abdominal examination, and standard investigations in accordance with the inclusion and exclusion criteria. Measurements of the traditional fetal biometric parameters were made using ultrasonography during the antenatal ultrasound examination.

The patients were divided into two groups: the study group (n = 100) received coamoxiclav in the form of a film-coated tablet called Megamox® (clavulanic acid 125 mg + amoxicillin 500 mg). produced in Saudi Arabia by AL-Jazeera Pharmaceutical and imported by Hikma Importation) twice a day for three days after birth, with a control group of one hundred patients not receiving postpartum antibiotics (n = 100).

As per the local hospital protocol, all women were positioned in the lithotomy position and then had their lower abdomen skin, thighs, and vagina sterilized with povidone iodine upon being moved to the labor room. Before head delivery, straight scissors were used to make an incision on the head's crowning and when the perineum was at its most stretched and ready to rupture. Local lidocaine was given at the episiotomy line and fourchette. Sim's speculum and two ovum forceps were used to inspect the whole vaginal wall and cervix after the full delivery of the baby, placenta, and membranes.

The extent of the episiotomy-cut wound was assessed before it was closed in layers (two muscle layers and the vaginal wall) with a continuous vicryl 2/0 suture that allowed for total homeostasis. With a subcuticular 2/0vicryl suture, the skin was sealed. Before the patient was sent to the post-natal ward for a two-hour surveillance of postpartum hemorrhage, a regular per-rectum examination was performed. Following a 12- to 24hour period, the patient was examined for any signs of local episiotomy hematoma, discomfort, or

excessive vaginal bleeding before being sent home with a follow-up card.

Follow up

Investigations, such as CBC, urine analysis, wound swabs for cultures, and chest x-rays, were sometimes required to confirm the diagnosis since infections might be diagnosed based on non-specific symptoms and indications.

The main result was an infection at the episiotomy site (edematous, erythematous, wound edge with pain, frankly purulent material, or wound dehiscence), and the secondary results included serious infectious complications like septic shock, bacteremia, systemic infection, fever (body temperature of 38 degrees Celsius or higher) occurring on any two occasions in the first 10 days postpartum, excluding the first 24 hours, puerperal infection, urinary tract infection, and endometritis.

Statistical Analysis

Numerical data that was normally distributed was statistically expressed as mean ± standard deviation (± SD), but data that was not normally distributed was expressed as median and range or inter-quartile range (IQR).

Results

Table 1: Demographic characteristics of study groups.

Demographic data	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Age (years) Mean±SD Range	 24.12±4.68 17-40	 24.43±4.71 17-39	 23.81±4.64 17-40	0.878	0.350
BMI [wt/ (ht) ^A2] Mean±SD Range	 24.81±3.10 19.1-33.3	 25.03±2.60 19.1-33.3	 24.58±3.53 22.6-33.3	1.030	0.311
BMI [wt/ (ht) ^A2] Mean±SD Range P2 PG	 19 (9.5%) 33 (16.5%) 8 (4.0%) 140 (70.0%)	 9 (9.0%) 20 (20.0%) 3 (3.0%) 68 (68.0%)	 10 (10.0%) 13 (13.0%) 5 (5.0%) 72 (72.0%)	2.152	0.542
GA "wks." Mean±SD Range	 39.01±1.09 37-41.6	 39.04±1.06 37-41.6	 38.98±1.13 37-41	0.150	0.699

There i s no statistically significant difference between groups according to demographic data, about Age (years), BMI [wt/ (ht) A2], Parity and GA "wks.", with p-value (P=0.350; P=0.311; P=0.542 ad P=0.699) (Table 1).

Table 2: Comparison between Group (1) and Group (2) according to Membrane rupture to delivery time

Membrane ruptures to delivery time (hrs.)	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Mean±SD	6.30±2.38	6.63±2.41	5.97±2.35	0.775	0.380
Range	0.08-23	0.08-23	0.17-21.5		

There is no statistically significant difference between groups according to membrane rupture to delivery time "hrs", with p-value (p=0.380) (Table 2).

Table 3: Comparison between Group (1) and Group (2) according to Admission to delivery time

Admission to delivery time (hrs.)	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Mean±SD	6.13±2.28	6.30±2.62	5.96±2.92	0.312	0.577
Range	1.25-25	1.25-25	1.25-21		

There is no statistically significant difference between groups according to admission to delivery time "hrs.", with p-value (p=0.577) (Table 3).

Table 4: Comparison between Group (1) and Group (2) according to Length of 1st stage of labour

Length of 1st stage of labour (hrs.)	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Mean±SD	4.05±2.35	4.00±2.05	4.09±2.02	0.074	0.786
Range	0.67-17.5	0.67-17.5	0.92-11.33		

There is no statistically significant difference between groups according to length of 1st stage of labour "hrs", with p-value (p=0.786) (Table 4).

Table 5: Comparison between Group (1) and Group (2) according to Length of second stage of labour

Length of second stage of labour (min.)	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Mean±SD	38.4±22.8	39.6±22.2	37.2±18.0	0.413	0.423
Range	4-120	5-120	4-120		

There is no statistically significant difference between groups according to length of second state of labour "hrs.", with p-value (p=0.423) (Table 5).

Table 6: Comparison between Group (1) and Group (2) according to complications

Complications	Total (n=200)	Group (1) (n=100)	Group (2) (n=100)	Test value	Pvalue
Wound Complication					
Erythematous Oedematous Purulent material	7 (3.5%) 3 (1.5%) 6 (3.0%)	3 (3.0%) 1 (1.0%) 2 (2.0%)	4 (4.0%) 2 (2.0%) 4 (4.0%)	1.715	0.788
Maternal Fever	0 (0%)	0 (0%)	0 (0%)	0.000	1.000
Serious Infectious complications	0 (0%)	0 (0%)	0 (0%)	0.000	1.000
Maternal Re-dmission	0 (0%)	0 (0%)	0 (0%)	0.000	1.000
Puerperal Infection	0 (0%)	0 (0%)	0 (0%)	0.000	1.000

Regarding complications from episiotomy wounds, the difference between the two groups was not statistically significant with a p-value of >0.05 in the presence of wound infection (erythematous, edematous, and purulent discharge).suggesting there was no statistically significant correlation between the preventative use of antibiotics and a decrease in the incidence of wound infections. There were no instances of maternal readmission, puerperal infections (endometritis, urinary tract infection), or severe infectious consequences (bacteremia, systemic infection, septic shock) in either group. In neither group did any occurrences of maternal fever occur (Table 6).

Table 7: Association between infection rate according duration of ROM in each group.

ROM	Infection rate				Total		x2	pvalue
	Non Infection		Infection					
	No.	%	No.	%	No.	%		
Group 1								
<18 hrs.	88	93.6%	6	100.0%	94	94.0%	0.407	0.523
>18 hrs.	6	6.4%	0	0.0%	6	6.0%		
Group 2								
<18 hrs.	85	93.4%	9	100.0%	94	94.0%	0.631	0.427
>18 hrs.	6	6.6%	0	0.0%	6	6.0%		
All group								
<18 hrs.	173	93.5%	15	100.0%	188	94.0%	1.035	0.309
>18 hrs.	12	6.5%	0	0.0%	12	6.0%		

x^2: Chi-square test for Number (%) or Fisher S exact test, when appropriate p-value >0.05 is insignificant

There is no statistically significant association between infection rate according ROM in each group and all group, with p-value (p>0.05).

Table 8: Association between infection rate according to Length of 1st stage of labour and Length of second stage of labour in each group

Stage	Infection rate				Test value	pvalue
	Non Infection		Infection			
	Mean	±SD	Mean	±SD		
Group 1						
Length of 1st stage of labour (hours)	3.91	1.60	5.38	2.21	0.526	0.470
Length of second stage of labour	0.65	0.39	0.68	0.24	0.862	0.355
Group 2						
Length of 1st stage of labour (hours)	3.96	1.89	5.40	2.81	3.291	0.073
Length of second stage of labour	0.58	0.26	1.06	0.55	0.599	0.441
All group						
Length of 1st stage of labour (hours)	3.94	1.28	5.39	2.86	2.390	0.124
Length of second stage of labour	0.62	0.30	0.91	0.30	0.308	0.579

Using: t-Independent Sample t-testfor Mean±SD; p-value >0.05 is insignificant

There is no statistically significant association between infection rate according to Length of 1st stage of labour and Length of second stage of labour in each group and all group, with p-value (p>0.05).

Discussion

In Our findings demonstrated that all research parameters were similar across study groups. Following an episiotomy, routine antibiotic use had little bearing on preventing wound complications, mother fever, puerperal infection, or readmission of the mother. In terms of demographic information, such as mother age, BMI, parity, gestational age at delivery, membrane rupture to delivery time, admission to delivery time, and duration of the first and second phases of labor, no differences were found between the research groups.

In 2022, Mohamed et al. conducted a comparison of the incidence of infectious morbidity among parturient women who had an uncomplicated vaginal delivery and were given regular local treatment alone vs a preventive course of oral antibiotics post-episiotomy. They came to the same conclusion as us—that prophylactic systemic antibiotic therapy is ineffective in preventing wound infection after an episiotomy. There was no statistically significant difference in the total wound complications after episiotomy between the antibiotic and non-antibiotic groups (7.8%-6.7%), as compared to our findings (6% vs 8%). There were no occurrences of atypical lochia or maternal fever reported on the second visit [11].

Up until 2021, the WHO, ACOG, and other organizations did not recommend antibiotic prophylaxis for women undergoing episiotomies. This makes it one of the contentious topics for obstetricians. According to ACOG Practice Bulletin No. 120 in 2011 and Bonet et al. in 2017, there was insufficient evidence to support routine antibiotic prophylaxis for episiotomy repair following a normal birth in order to reduce maternal or fetal infectious morbidity. As a result, the Cochrane Library recommended conducting more RCTs in 2016. [12,13].

Although exact statistics on the prevalence of episiotomy in Egypt are unavailable, both public and private institutions often perform the procedure. The primary conclusions of our research show that the incidence of infection in patients with episiotomies after a typical vaginal birth of low-risk parturient women was not significantly reduced by the administration of preventive antibiotics. Additional-

ly, a significant proportion of women would likely be exposed to antibiotics needlessly because to the very high rate of episiotomies.

According to Ramirez et al.'s 2020 report, the CDC advised against prescribing unnecessary antibiotics because they can have a number of negative effects on the mother and the newborn, including upsetting the microbiota, antibiotic resistance, an increased risk of drug poisoning, hypersensitivity reactions, and needless costs [14].

In line with the current investigation, Garala and Nambiar (2019) found no statistically significant differences between the two groups that got or did not receive antibiotics after an episiotomy with relation to puerperal pyrexia, wound infection, and length of hospital stay [15].

Apart from instrumental vaginal delivery, Akram et al. (2019) and Tandon and Dalal (2018) reported using various antibiotic types, single or multiple doses, in different studies, such as amoxi-clavulinic acid, cefoxine metronidazole combination, and chloramphenicol. They all concluded that there was no benefit of antibiotic prophylaxis after episiotomy repair [16, 17].

Despite their recommendation, Knight et al. (2019) link the high infection rate in the control group to the possibility of microorganisms entering the genital tract during operative vaginal delivery. They also link this procedure to longer labor, more vaginal examinations, bladder catheterization prior to the procedure, more perineal lacerations, and the use of episiotomies, all of which increase the risk of infection [18].

The incidence of episiotomy site infection was compared in two groups of primiparas with and without taking prophylactic antibiotics after a normal vaginal delivery. Goodarzi et al.'s 2020 RCT, which contrasted with our study, found that the antibiotic group had a better wound healing as indicated by a lower healing score than the placebo group. However, they used chromic sutures and midline episiotomy, both of which enhance the risk of infection [19].

In 2022, Sirilak et al. set out to ascertain the prevalence of postpartum infections as well as the consequences and variables linked to antibiotic prophylaxis in women who had vaginal deliveries. They agreed with us, backed the practice standards, and gave the medical staff peace of mind that no more than 10% of women giving birth naturally would need the use of antibiotics. The incidence of postpartum infection did not significantly vary between individuals who received antibiotics and those who did not. There was no statistically significant difference in the number of postpartum infections reported between 11 of 792 women without antibiotic prophylaxis and 3 of 117 individuals receiving them [20].

The risk of urinary tract infections, wound infections, and length of hospital stay in women treated with antibiotics was not different from that of those who received a placebo or no antibiotics, according to a 2017 systematic review by Bonet et al. on the use of antibiotics to prevent infection in women after delivery [13].

In the 2018 research by Tandon and Dalal, the infection-related symptoms were 0.7 and 2% in the groups receiving antibiotic treatment and the untreated group, respectively.

Infection symptoms did not vary statistically significantly between the two groups ($p<0.622$) [17].

In 2016, a research conducted in Sirilampang, Thailand, the results of complications in postpartum sepsis were not different between the groups receiving antibiotic treatment and those not receiving any [21].

According to Sangprappai's 2011 report, neither group had postpartum infections, with 57 women receiving amoxicillin treatment and 56 not receiving it. In this investigation, the criteria for assessing infections were comparable. These trials' findings demonstrated that, in women who gave birth normally,

the outcome of postpartum infection did not change depending on whether or not antibiotics were used. Therefore, unless a patient has a third or fourth degree tear, antibiotic prophylaxis should not be used to prevent postpartum infections in women who give birth vaginally [22].

According to a 2017 study by Bonet et al, there was no statistically significant difference in the use of antibiotics in episiotomy for infected wounds between the groups receiving antibiotic treatment and those not [20].

According to WHO recommendations from 2015, women with episiotomy lesions should not get antibiotic prescriptions as an empirical therapy for postpartum infections [23].

the American College of Obstetricians and Gynecologists does not advise against using antibiotics to prevent postpartum infections [24].

Conclusion

It is ineffective to prevent wound infection after an episiotomy by administering a prophylactic systemic antibiotic. In conclusion, even though it's a fairly prevalent practice in low-income countries, giving oral antibiotics to low-risk patients after their episiotomies are unnecessary and have not been shown to lower the incidence of postpartum infections.

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The Value of Ultrasonographic Sliding Sign in Prediction of Intra-Abdominal Adhesions in Pregnant Women Undergoing Cesarean Section Following Abdominopelvic Surgery: A Prospective Study

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Running title

Sliding Sign for Intra-Abdominal Adhesions

Mobile:

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Abstract

Objective: The current evidence is inconclusive regarding the utility of sliding sign for prediction of intra-abdominal adhesion. In the present study, we assessed the diagnostic accuracy of negative sliding sign for prediction of intra-abdominal adhesion in pregnant women scheduled for Cesarean section (CS) and had a history of previous abdominopelvic surgery.

Methods: A prospective, observational, study was conducted on 158 full-term pregnant women who were candidates for elective cesarean section.

Results: A total of 16 (10.2%) women had negative sliding sign and 8.9% had marked adhesions. The positive sliding sign accurately detected 138 (95.8%) out of 144 patients with no or minor adhesions, while the negative sliding sign correctly recognized 10 (71.4%) out of 14 patients with significant adhesions during the CS treatment. The sliding sign demonstrated a sensitivity of 71.4%, specificity of 95.8%, positive predictive value (PPV) of 62.5%, and negative predictive value (NPV) of 97.1% in predicting significant intra-operative adhesions. In contrast, patients who did not exhibit a sliding sign had a higher probability of suffering from intra-operative bleeding, a significant decrease in hemoglobin levels of more than 3 g/dl, visceral injury, and a longer delivery time ($p < 0.001$).

Conclusion: In conclusion, our findings highlight that the negative sliding sign can be used for prediction of severe intra-abdominal adhesions; the negative sliding sign is an accurate, reproducible, and easily accessible method for prediction of adhesions in clinical setting. Moreover,

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patients with negative sliding sign appears to have higher risk of prolonged delivery, intra-operative bleeding, and visceral injury.

Keywords: Intra-abdominal adhesion; Cesarean section; Maternal outcomes; Sliding sing; Ultrasound.

Abbreviations

CS: Cesarean section

STROBE: STrengthening the Reporting of OBservational studies in Epidemiology

BMI: Body mass index

TAUS: Trans-abdominal ultra-sonography

Key massage

- Sliding sign can be used for prediction of intraabdominal adhesions
- The assessment is feasible during the third trimester.
- Routine assessment of sliding sing is warranted to accurately anticipate the complexity of the procedure and properly plan for it..

Introduction

Cesarean section (CS) is the most common obstetric surgery, with an increasing prevalence of the US by 50% over the last decade[1]. CS is mostly performed according to fetal indications but can also lead to some significant maternal and fetal complications[2]. Hemorrhage, hysterectomy, infection, bladder, and bowel injury are the main complications of CS[3–6]. Furthermore, intra-abdominal adhesions due to surgical duration and the perinatal adverse outcome associated with the postpone of the neonate delivery in the case of an urgent CS are among the most critical complications of CS[7,8].

Intraabdominal adhesion is an irregular bond between the anatomic structural surfaces, with a varying severity after repeated intraabdominal or pelvic procedures[9]. The implications of these kinds of abnormal associations include bowel obstruction, persistent abdominal and pelvic pain, the necessity for re-intervention, ectopic pregnancy, miscarriage, and accidental damage or failure to the organ throughout surgery [10,11].

Some investigators have suggested abdominal scar characteristics and surgical history as potential indicators for adhesion presence and severity, due to the lack of a reliable non-invasive method for preoperative identification of intraabdominal adhesions[12,13]. Nevertheless, these techniques are not reproducible, and the history of prior surgery also maybe does not exist. On the other hand, it has been shown that the sonographic sliding organ sign has high predictive power for detecting pelvic adhesions in women with chronic pelvic inflammation, endometriosis, and infra-umbilic adhesions before the laparoscopic procedure [14,15]. The idea behind this technique is to assess the relative motion between the abdominal and uterine walls by ultrasonography[15]. A negative sliding sign showed a significant role in predicting the severe intraabdominal

adhesions owing to its high specificity[16]. In this study, we aimed to investigate the predictive value of ultrasonographic sliding sign towards the intraabdominal adhesions in pregnant women undergoing CS following abdominopelvic surgery.

Materials and Methods

We prepared the present manuscript in concordance with the recommended standards of STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement[17]. The study's protocol was approved by local ethics committee of Kasr-Alainy University hospital.

Patients and Setting

We conducted a prospective, observational,

study at the Obstetrics & Gynecology Department, Kasr-Alainy hospital between March 2019 and September 2019. The study involved 158 pregnant women who were elected for CS. All Patients were informed about the study and were required to give verbal consent for study participation and to sign a written consent for conduction CS. The inclusion criteria targeted full-term pregnant with prior history of abdominopelvic surgery. Patients with a body mass index (BMI) of more than 40Kg/m2, abnormal placental invasions, or emergent CS were excluded.

Sample size calculation was based on the sensitivity of the sliding sign detected by ultrasound. Literature indicated that the sensitivity of the sliding sign in predicting intra-abdominal adhesion after Cesarean section ranged from 56% to 76.2%, with an average of 66.1%. Assuming that the prevalence of intra-abdominal adhesions after CS is 38%(8). These assumptions estimated a sample of 158 full-term pregnant women to detect the main effect with 80% power setting type I error rate of 5%. Calculations were done using Flahault et al's equation[18].

Data Collection and Sliding Sign Assessment:

Each participating woman was subjected to a full history taking, clinical examination, and routine laboratory investigations. The operative time, pre and postoperative hemoglobin levels, and the incidence of bladder and/or bowel injury were registered as well.

Trans-abdominal ultra-sonography (TAUS) was conducted using Samsung Ws80a systems during the preoperative examination, using the real-time TAUS pelvic sliding sign as described by Drukker et al[16]. The sonographers were blinded to the type of previous surgery to interpret objectively the TAUS sliding sign.

The patient was advised to take deep breaths, emphasizing her respiratory motions, while the sonographer recorded a video clip in a mid-sagittal plane positioned to the side of the umbilicus, focusing on the area below the umbilicus. The purpose was to assess if the structure moved smoothly in relation to nearby structures. In order to be classified as sliding, the structures needed to exhibit smooth movement of their surfaces (indicating positive sliding). Negative sliding was identified when no movement of the structures was seen[19].

Assessment of Adhesion

Patients underwent a lower segment cesarean section (CS) 24 hours following the TAS assessment. Surgeons directly observed and offered a full description of any adhesions and unintentional harm. The adhesions were classified according to a standardized grading system into: Grade 0: No adhesions were found. Grade 2: Moderate or thick adhesions were observed. Grade 3: There was no free space between the anterior

abdominal wall and the uterine wall[20]. The surgeons were blinded to the TAUS sliding sign results.

Study's Outcomes

The primary outcome of this observational prospective study was the predictive accuracy of sliding signs for intraabdominal adhesions using TAUS done to full term pregnant after CS operations. Secondary outcomes included the associations between negative sliding signs and sever intraabdominal adhesions, operative times (skin incision to delivery of the baby), hemoglobin drop, and bladder or bowel injury.

Statistical analysis of the collected data

Data were managed using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Quantitative data were summarized using mean, standard deviation, median, and range. Regarding qualitative data, frequency (count) and relative frequency (percentage) was calculated. Normally distributed continuous variables were tested using independent sample t-test. Mann-Whitney test was

performed on the continuous variables that not following the normal distribution. For comparing categorical variables, Chi-square ($\chi 2$) and Exact tests were conducted. A p value less than 0.05 was considered statistically significant (two-sided testing).

Results

The mean age of the included women was 30.6 ±4.04 years and the mean BMI was 30.9 ± 2.5 kg/m2. The average gestational age of the participants was 38.2 ± 2.3 weeks. Of the included patients, 90.5% were multi-paras. The vast majority of the women (77%) had a history of previous CS, 14% underwent appendectomy before, 6% had done open myomectomy before, and 3 % experienced previous exploratory laparotomy. The frequency of positive sliding sign was 89.2% among the recruited patients. Among the three categories of adhesion severity, 82.9% of the involved participants had no adhesions after CS procedure, while 8.9% had marked adhesions. Furthermore, 3.8% of the patients were complicated with a visceral injury. Only 2.5% of the study cohort complicated with HB drop> 3g/dl (Table 1).

Overall, the positive sliding sign was correctly identified in 138 (95.8%) out of 144 patients who had no or mild adhesions on CS procedure while negative sliding sign was correctly identified 10 out of 14 (71.4%) patients who had marked adhesions on CS procedure (Figure 1). Thus, negative sliding sign yielded a sensitivity of 71.4%, specificity of 95.8%, positive predictive value of 62.5%, and negative predictive value of 97.1% for detection of marked adhesions.

Regarding delivery time, patients with negative sliding signs recorded significantly longer delivery time than positive patients ($p < 0.001$). Also, patients with negative signs were more liable to visceral injury (25.0%) compared to patients with positive sign (25.0% vs 1.4%, $p <0.001$). According to the site of injury, the incidence of bladder injury among patients with positive sign was 1.4 % (2 out of 142) and 18.7 % (3 out of 16) among patients with negative one and incidence of intestinal injury among

patients with negative sign was 6.25% (1 out of 16), no intestinal injuries were recorded in patients with positive sign (Table 2).

Discussion

The current evidence is inconclusive regarding the utility of sliding sign for prediction of intra-abdominal adhesion. In the present study, we assessed the diagnostic accuracy of negative sliding sign for prediction of intra-abdominal adhesion in pregnant women with a history of previous abdominopelvic surgery. The negative sliding sign achieved fair diagnostic accuracy for prediction of severe intra-abdominal adhesions. In addition, patients with negative sliding sign had significantly higher incidence of prolonged delivery, intra-operative bleeding, and visceral injury.

Intraabdominal adhesion is a major concern in women undergoing CS, especially among those with a history of abdominopelvic surgery; previous reports indicated that intraabdominal adhesion significantly increased the risk of intra and postoperative complications such as bowel obstruction, excessive intraoperative hemorrhage, prolonged operative time, and accidental damage or failure to the organ throughout surgery[21–23]. Thus, it is imperative to accurately predict intra-abdominal adhesion in at-risk women; nonetheless, established predictors, such as abdominal scars, revealed low diagnostic accuracy and poor reproducibility[12,13]. Thus, recent reports suggested the use of ultrasound-based techniques as a simple, non-invasive, predictors of intra-abdominal adhesion[24]. In the present study, we demonstrated that negative sliding sign achieved fair diagnostic accuracy for prediction of severe intra-abdominal adhesions; negative sliding sign

yielded a sensitivity of 71.4%, specificity of 95.8%, positive predictive value of 62.5%, and negative predictive value of 97.1% for detection of marked adhesions.

These findings are in line with previous report by Drukker et al.[25], who showed that the negative sliding sign achieved a sensitivity and specificity of 56 and 95%, respectively, for prediction of severe adhesion in women with history of CS. In another report on 59 women, the negative sliding sign achieved sensitivity and specificity of 76.2% and 92.1%, respectively, for detection of high risk adhesions[19]. Notably, Ayachi et al.[26], reported higher diagnostic performance of sliding sign among women with previous abdominopelvic surgery, with a sensitivity and specificity of 96.3% and 92.6%, respectively. The sliding sign demonstrated good diagnostic accuracy for prediction of intraabdominal adhesion for in women undergoing other procedure than CS as well[27].

Factors, such as prolonged delivery time and excessive bleeding, adversely affect the maternal and neonatal outcomes of CS; while intraabdominal adhesion is a risk factor for prolonged delivery time, intraoperative bleeding, and visceral injury[28]. In the present study, we demonstrated that the negative sliding sign correlated significantly with prolonged delivery, intra-operative bleeding, and visceral injury. These findings were in agreement with reports by Drukker et al.[25] and Ayachi et al.[26].

Our findings have important clinical implications. Based on our findings, negative sliding sign is an accurate, reproducible, and easily accessible method for prediction of adhesions in clinical setting. The assessment is feasible during the third trimester in pregnancy and the BMI do not appear to affect its result[19]. Thus, routine assessment of sliding sing in at-risk women is warranted preoperatively. In at-risk women, surgeons can accurately anticipate the complexity of the procedure and properly plan for it. The surgeon can even refer the case to specialized, equipped, center with multidisciplinary team or experienced surgeon; especially that our results indicated the negative sliding

sign correlated significantly with intra and postoperative complications such as prolonged delivery, excessive hemorrhage, and visceral injury.

We acknowledge that the present study has some limitations. The significant association between negative sliding and intra-abdominal adhesions was not adjusted according to type of previous abdominopelvic surgery or the number of adhesions owing to the small number of patients with negative sliding sign. Another limitation is the use of surgeon-based score for grading of intra-abdominal adhesions, which may lack consistency between surgeons. We did not include morbidly obese women in our study as well; those the impact of obesity on the results of ultrasound assessment was not studied in the present report.

In conclusion, our findings highlight that the negative sliding sign can be used for prediction of severe intra-abdominal adhesions; the negative sliding sign is an accurate, cheap, easily accessible, and reproducible method for prediction of adhesions in clinical setting. Moreover, patients with negative sliding sign appears to have higher risk of prolonged delivery, intra-operative bleeding, and visceral injury. Based on the results of our study, we recommend the detection of the simple, non-invasive, sliding sign prior to elective CS in order to discriminate between high and low risk intraabdominal adhesions in patients with history of previous abdominopelvic surgery; this practice may decreases the risk of complications such as bladder, bowel injury, and hemorrhage. Nonetheless, the findings of the present study should be further investigated.

Disclosure of interests

All authors confirm no financial or personal

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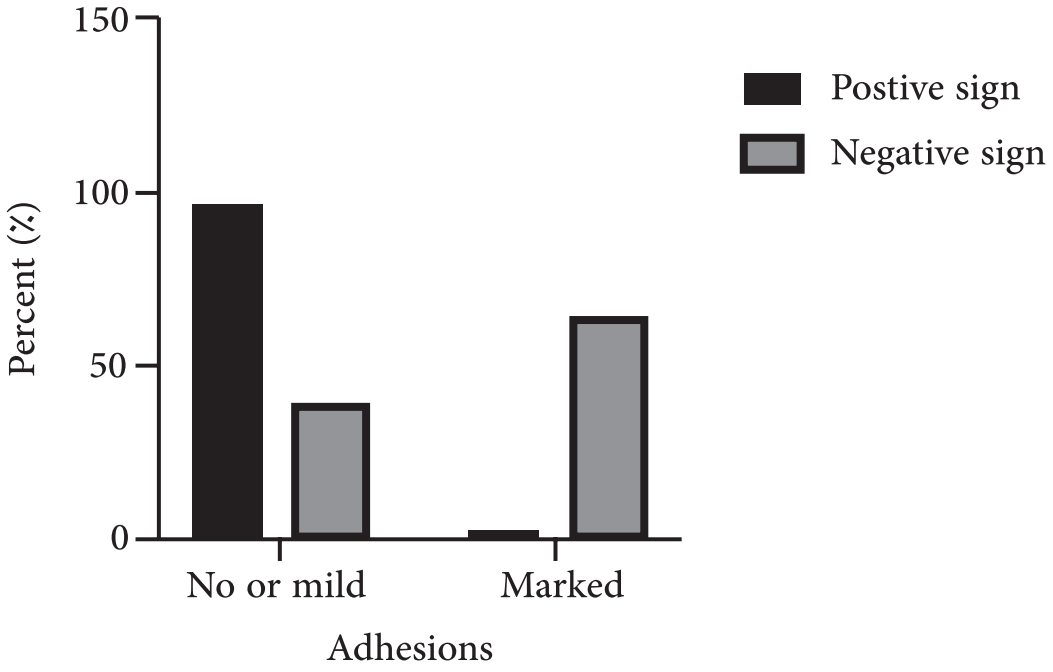
Table 1: Outcomes of study participants.

Outcome	Study cohort (n = 158)
Sliding sign, n (%)	
Positive	142 (89.2)
Negative	16 (10.2)
Intraoperative adhesions, n (%)	
No	131 (82.9)
Mild	13 (8.2)
Marked	14 (8.9)
Hb drop (g/dl) , n (%)	
<1	45 (28.6)
1-2	101 (63.9)
2-3	8 (5)
>3	4 (2.2)
Visceral Injury, n (%)	
No	152 (96.2)
Yes	6 (3.8)

Table 2: the relationship between sliding sign and adhesions in the study patients.

Parameters	Positive (n= 142)	Negative (n=16)	P value
Adhesions, n (%)			
No or mild	138 (97.1)	6 (37.5)	<0.001
Marked	4(2.9)	10(62.5)	
Delivery time, Median (IQR)	8 (7 – 9)	18 (10.5 – 20)	<0.001
Hemoglobin drop, n (%)			
<1g/dl	25 (17.6)	1 (6.25)	
1-2g/dl	111 (78.2)	9 (56.25)	<0.001
2-3g/dl	6 (4.2)	2 (12.5)	
>3g/dl	0 (0.0)	4 (25.0)	
Visceral injury, n (%)			
Yes	2 (1.4)	4 (25.0)	<0.001
No	140 (98.6)	12 (75.0)	

Figure 1: percentage of adhesion



Three-dimensional ultrasound assessment of endometrial compaction before embryo transfer, is it worthy?

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Abstract

Background & Objective: Endometrial thickness and vascularity are commonly used predictors of endometrial receptivity. We aimed to evaluate whether the three-dimensional ultrasound assessment of changes observed in the endometrium on the day of FET compared with that on the day of initiation of progesterone has an impact on the clinical pregnancy rates in FET cycles.

Materials and Methods: A prospective study was performed in a specialized fertility center from February 2021 to February 2023. The study included 150 FET cycles in which endometrial preparation was done with hormonal replacement therapy. Using 3D ultrasound, the alterations in endometrial parameters (thickness, volume, and endometrial blood flow indices) among the day progesterone was initiated and the day FET were evaluated were compared. An analysis was conducted on the relationship among endometrial changes and clinical pregnancy rates (CPR).

Results: Overall, 161 participants were enrolled in the current study, 11 were excluded, and 150 were included into statistical analysis. Among those 150 patients, 102 were pregnant (68%) and 48 were not pregnant (32%). Baseline demographic, clinical and cycle characteristics were matched between groups with no significant differences detected. Clinical pregnancy rates among endometrial thickness change groups (compaction, expansion, and stable groups) were 48%, 46%, and 28% respectively. Clinical pregnancy rates among endometrial volume change groups (decrease, increase and stable groups) were 82%, 2% and 18% respectively .In addition, logistic stepwise analysis for the effect of changes in endometrial indices on clinical pregnancy rate revealed that endometrial volume change percent was the only significant variable predicting CPR ($P<0.0001$) and CPR was not significantly associated with endometrial thickness change ($P=0.961$) or endometrial blood flow change. ROC curve showed that endometrial volume change percent with cut off value of 10.44% had 72.55% sensitivity and 77.08% specificity for prediction of clinical pregnancy rate.

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Conclusion: The endometrial volume changes after progesterone administration was the only significant independent predictor of clinical pregnancy rate in FET cycles. Furthermore, a change in the endometrial volume of 10.44% was associated with significant improvement in clinical pregnancy rates of FET cycles with artificial endometrial preparation.

Keywords: Endometrium, thickness, volume, 3D ultrasound, frozen-thawed embryo transfer, clinical pregnancy rate.

Introduction

Up until now, the majority of fertility clinics had favored the "freeze-all" approach, which was prompted by developments in embryo cryopreservation and thawing, in addition to the prohibition of ovarian hyperstimulation syndrome risk. The success of a pregnancy in frozen/thawed embryo transfer (FET) cycles is significantly influenced by two critical factors: the embryo morphology and the endometrial receptivity. (1).

In order to create an environment conducive to embryo implantation, there are certain modifications that must occur in the infrastructure vascular network, surface epithelium, and expression levels of glycoproteins, integrins, chemokines and receptors. These conditions exist for a brief period of time (around 5 days) and are referred to as "the window of implantation" (2,3).

An assortment of methodologies have been utilized in the examination of endometrial receptivity. The assessment of endometrial receptivity has been conducted noninvasively via transvaginal ultrasound (TVU), employing various sonographic parameters including endometrial pattern (A=triple-line, B=intermediate isoechogenic, and C=homogenous hyper-echogenic), endometrial thickness (EMT), and endometrial blood flow indices (4).

A number of trials have investigated the correlation between endometrial thickness at the time of embryo transfer (ET) and on the day of triggering and pregnancy outcome. While certain investigations indicate a positive correlation, others have produced inconclusive findings. A lower limit of EMT <8 mm in fresh and <7 mm in FET cycles was identified by Liu et al. in their analysis of over 40,000 ET cycles; such cycles resulted in lower clinical pregnancy rates (5). Conversely, El-Toukhy et al. discovered that an upper limit exceeding 13 mm was associated with a diminished rate of pregnancy and proposed the existence of an ideal range for EMT in order to attain a higher rate of pregnancy (6). Nine prospective and twenty-one retrospective studies encompassing a total of 88,056 cycles were evaluated by Gao et al. in a meta-analysis. The researchers concluded that a lower EMT was associated with lower pregnancy rates (7), as opposed to a higher EMT. A potential relationship among EMT and implantation rate could exist; however, due to the complexity of this process, a solitary ultrasound measurement might not be sufficient to confirm this relationship (8).

Haas et al. introduced the novel notion that endometrial compaction, which they defined as the decrease in endometrial thickness from the day of frozen embryo transfer (FET) to the last day of the estrogen proliferative phase, could potentially be a more dependable indicator of pregnancy outcomes than a single endometrial thickness measurement on FET day(9). Four subsequent studies examined the effect of endometrial compaction on FET outcomes and produced conflicting results. A positive relationship was observed in two trials conducted by the same group (9, 10); nevertheless, endometrial compaction failed to increase pregnancy rates in two other trials (3, 11). Based on a review of the existing research, it appears that additional researches are necessary to illuminate and reach a more definitive conclusion in this area.

The volume and vascularity of the receptive endometrium may be evaluated via 3D ultrasound as one method of assessment. It has

been demonstrated that endometrial and subendometrial vascularization increases during the proliferative phase, reaches its maximum three days prior to ovulation, and declines to its minimum five days after ovulation, as determined by 3D power Doppler. There is evidence to suggest that the extent of perfusion changes could potentially influence endometrial receptivity (12).

In order to determine whether the clinical pregnancy rates in FET cycles are affected by the three-dimensional ultrasound assessment of endometrial changes among the day of progesterone initiation and the day of FET, this prospective study sought to determine whether this comparison is significant.

Materials and Methods

This prospective observational study was conducted between February 2021 and February 2023 at a specialized fertility center. Eligible patients were screened on the initial day of endometrial preparation. Following a comprehensive review of the inclusion and exclusion criteria, informed consent was obtained from all participants.

Inclusion criteria:

Females who are younger than forty years old, have a body mass index (BMI) below 30 kg/m2, and are undergoing their initial FET cycle in which two high-quality blastocysts are transferred.

Exclusion criteria:

Severe male factor, uterine malformation, recurrent miscarriages, implantation failures, endometriosis, adenomyosis, and inadequate progression of endometrial thickness.

Complete medical evaluation (body mass index ,age and duration of infertility) and basal hormonal profiles (FSH, LH, E2, AMH, and progesterone) were obtained from each candidate.

Sample size calculation

A review of previous research (10) revealed that pregnancy rates were significantly higher during cycles in which the endometrial lining was compacted as opposed to those in which it was not compacted. 54.9 percent of pregnancies occurred in the compaction group, compared to 31% in cycles where the endometrium did not compact. Statistical software determines the minimum sample size, and version 6 of the sample size program divides 150 subjects into two equal groups. The trail has an 80% power and a 95% confidence level.

Treatment Protocol

All participants underwent endometrial preparation in conjunction with hormone replacement therapy (HRT), which entailed the daily administration of oral estradiol valerate (6 mg) commencing on the first day of each cycle. If endometrial thickness ≥7 mm was observed 12-14 days after estradiol exposure, progesterone supplementation was initiated in the form of progesterone in oil 100 mg intramuscular injection and progesterone vaginal suppository twice daily, while estradiol valerate was maintained at the same dose. In the event that the endometrial thickness failed to attain 7mm, the daily estradiol dosage was escalated to 8 mg until the desired EMT was achieved. In cases where pregnancy tests were positive, progesterone and estradiol valerate continued to be administered until the day of the pregnancy test, and in those cases, until the tenth week of gestation.

Endometrial Assessment

Endometrial thickness (i.e., the maximum endometrial thickness in a longitudinal plane, observed on both sides of the midline) was quantified utilizing two-dimensional ultrasound.

Using power Doppler, the ultrasound was converted to three-dimensional mode. The endometrial volume estimation was performed using the VOCAL program, while three indices of endometrial blood flow were generated using the "histogram facility": the

flow index (FI), vascularization index (VI) and vascularization flow index (VFI).Manually, the endometrial area in the coronal plane was determined. The subendometrial region was incorporated; it was defined as the outermost layer up to five mm from the junction of the endometrium and myometrium. All patients underwent two measurements, and the mean values were calculated utilizing the Voluson E10 probe model RIC5-9-D from GE Healthcare.

Changes were observed during the ultrasound endometrial assessment performed twice: on the day progesterone was initiated (P+0) and on the day the blastocyst embryo was transferred (P+6). Variations in the endometrial thickness among the two observations were classified as "compaction," "expansion," or "no change." The two endometrial volume measurements were classified as "no change," "increase," or "decrease."

Evaluation of embryo morphology and embryo transfer

The evaluation of blastocyst embryos was conducted in accordance with the criteria outlined by the ESHRE (13). Embryo vitrification was performed a period of five days subsequent to insemination (14). Patients who had embryos of the highest quality (A and AB) that were available for transfer participated in the present study. All participants underwent ET utilizing the identical catheter type (Wallas catheter, Queensland, Australia).On the sixth day following progesterone administration, a maximum of two high-quality frozen embryos were thawed and subsequently transferred at the blastocyst stage, contingent upon the age of the women.

The association among changes in endometrial volume, thickness and blood flow indices and clinical pregnancy rates (as determined by vaginal ultrasound observation of a gestational sac with a heartbeat) was the primary outcome.

Statistical Analysis

The statistical analysis was conducted using version 21.0 of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). Using the chi-square test, the categorical variables of the groups were compared. The non-normal distribution of all continuous variables in this trail was determined by the Kolmogorov–Smirnov test; consequently, the Kruskal–Wallis test was employed to compare the groups. The purpose of the multivariable logistic regression was to identify the significant independent predictors of clinical pregnancy. A level of significance of $P<0.05$ was established.

Results

In total, 161 individuals were registered for the present study; of these, 11 were excluded and 150 were deemed suitable for statistical analysis. One hundred two (68%) of the 150 patients were pregnant, while 48 (32%) were not pregnant (Fig. 1). In relation to baseline demographic, clinical, and ICSI cycle characteristics, non statistically significant differences were identified in the following areas: body mass index, age , basal hormonal profile, duration and cause of infertility, estrogen supplementation duration, and number and quality of embryos transferred (Table 1).

Comparisons among pregnant and non-pregnant groups with respect to various 3D endometrial parameters were presented in Table 2. Significant differences were obtained between the pregnant and non-pregnant groups regarding the endometrial volume difference between the days of frozen ET and progesterone initiation (P=0.000).

Clinical pregnancy in endometrial thickness change groups: compaction, expansion, and stable groups were 48%, 46%, and 28% respectively (Fig.2) .CPR in endometrial volume change groups: decrease, increase and stable groups were 82%, 2% and 18% respectively (Fig.3)

In the stepwise logistic regression model, all potential confounding variables were incorporated to ascertain the presence of any significant factors that are independently

associated with the clinical pregnancy. The findings of the analysis indicated that cycles involving an endometrial volume change during the transfer of frozen embryos in artificially prepared endometrium were significantly and independently associated with an improvement in clinical pregnancy rates (P<0.0001) (Table 3).

Table 4 showed validity of endometrial volume change percent in prediction of clinical pregnancy outcome among the studied groups. ROC curve showed that endometrial volume change percent with cut off value of 10.44% had 72.55% sensitivity and 77.08% specificity for prediction of clinical pregnancy rate.

Discussion

Endometrial proliferation ceases when serum progesterone reaches its maximum level (approximately three days after ovulation); therefore, endometrial mass (EMT) should not change, as blood vessels and the glands have fully developed; this is consistent with increased endometrial density rather than height. Consequently, endometrial compaction may serve as a more reliable physiological indicator of endometrial receptivity. While there have been speculations that persistent endometrial growth during the secretory phase (as a result of progesterone resistance) could indicate an unsuitable environment for embryo implantation, the available evidence is limited and further research is required to investigate this matter (3, 15).

So, the current setting aimed to investigate which category of endometrial volume change: compaction, expansion or stable groups had associated with a superior clinical pregnancy rate in FET cycles.

We reported that change in endometrial volume was the only significant independent predictor of clinical pregnancy in frozen-thawed ET cycles. Furthermore, endometrial volume change after progesterone initiation with cut off value of 10.44% was associated with a significant improvement in clinical pregnancy rates (P<0.0001). Meanwhile, alterations in endometrial stripe thickness and blood flow were not helpful in prediction of pregnancy outcomes of frozen ET cycles.

The effects of endometrial compaction on live birth rates (3) and ongoing pregnancy (9-11) in FET cycles were evaluated in four prior studies. Haas et al. initially reported that endometrial compaction cycles were associated with a greater rate of ongoing pregnancies. Furthermore, there was a significant rise in the rate of ongoing pregnancies as the percentage of compaction rose (9). Significantly higher than the 16.6% rate observed by Riestenberg et al. (3), the rate of decreased endometrium thickness (endometrial compaction) in their cohort (9 was 42.4%). Zilberberg et al. (10) reported comparable results in their second study. The utilization of artificial endometrial preparation and endometrial compaction on the day of ET resulted in a higher pregnancy rate (43.1%) during single euploid FET cycles. A document was found to indicate an endometrial compaction rate of ≥5% (10). Two subsequent trials, on the other hand, generated controversial findings (3, 11). The clinical pregnancy rate was higher in cycles with endometrial expansion subsequent to progesterone initiation for both types of endometrial preparation, according to a trial by Bu et al. (11) involving more than 3000 blastocyst FET cycles and a significant sample size. In the cohort under study, the incidence of endometrial compaction was 19.6% during the medicated cycle and 26.2% during the natural cycle (11). Following this, a group of 259 single euploid medicated FET cycles were evaluated by Riestenberg et al. They reached the conclusion that endometrial thickness did not decrease in 83.4% of the cycles examined; additionally, this result did not function as a predictive factor for outcomes in in vitro fertilization (3).

Divergent outcomes observed in these investigations might be ascribed to differences in the protocols for endometrial preparation and

the scheduling of time for the two ultrasound examinations (3). The ultrasound performed at the end of the E2 phase was trans-vaginal in the first two trials, while the ultrasound performed on ET day was trans-abdominal. All ultrasound examinations, including the current one and two others, were performed transvaginally, and endometrial compaction was not observed in the majority of cycles. The present study, along with all prior investigations (9, 11), conducted the second phase of EMT measurement at the moment of ET. However, Riestenberg et al. (3) deviated from this pattern by conducting the measurement one day prior to ET.

Several studies (16, 17&18) examined endometrial volume as an alternative to endometrial thickness in order to forecast endometrial receptivity and reached the conclusion that EV cannot be utilized to predict pregnancy outcomes, irrespective of whether it is computed on the day of ovum pickup (17) or the β-hCG day (18). The primary indicator of endometrial volume is the dimensions of the uterus, which may account for this result. Monitoring changes in endometrial volume could therefore eliminate the influence of uterine size and provide a more accurate reflection of endometrial changes. Similar to previous research, variations in endometrial thickness were not predictive of pregnancy outcomes in the present study (19, 20). Endometrial thickness measurement was significantly impacted by endometrial peristalsis (21, 22), with its manifestation being especially conspicuous on the day of P initiation. This may provide an explanation for the inability of EMT measurements to precisely reflect endometrial changes. By measuring endometrial volume, which has a greater predictive capacity for clinical pregnancy rate, this effect was eliminated.

Our research demonstrated that 3D power doppler imaging of endometrial blood flow changes did not provide any predictive value for pregnancy rates in HRT -prepared cycles. The findings of this trial were in line with those of more recent research (23, 24).

Strength points

First, our research established that EV fluctuations were a significant predictor of IVF success. In contrast to other three-dimensional parameters such as VFI, FI and VI, EV is straightforward and convenient to acquire in clinical practice. Furthermore, in comparison to alternative methods of endometrial receptivity assessment, EV is a cost-effective and non-invasive technique

Because we were investigating endometrial changes during HRT-frozen ET cycles, we also minimized the number of ultrasound examinations performed and abstained from daily ultrasound inspections throughout the cycle.

In the third step, a logistic stepwise adjustment was performed to account for additional confounding variables that may have influenced the clinical pregnancy rates. Furthermore, we utilized the ROC curve to establish the threshold value of endometrial volume change percent in order to accurately predict clinical pregnancy rates with exceptional sensitivity and specificity.

Limitations

No inquiry was conducted into the rates of ongoing pregnancies or live births.

The current body of evidence in this field remains constrained. Hence, the determination of whether to cancel the ET or modify the endometrial preparation protocol in response to an endometrial volume change observed on the FET day is not possible. Therefore, additional prospective studies utilizing a more significant sample size are warranted in order to assess the predictive value of endometrial volume change in relation to FET outcomes during both programmed and natural cycles.

Conclusions

In FET cycles, only changes in endometrial volume subsequent to progesterone administration were found to be a significant inde-

pendent predictor of the clinical pregnancy rate. In addition, a 10.44% change in endometrial volume was found to be significantly associated with improved clinical pregnancy rates in FET cycles utilizing artificial endometrial preparation.

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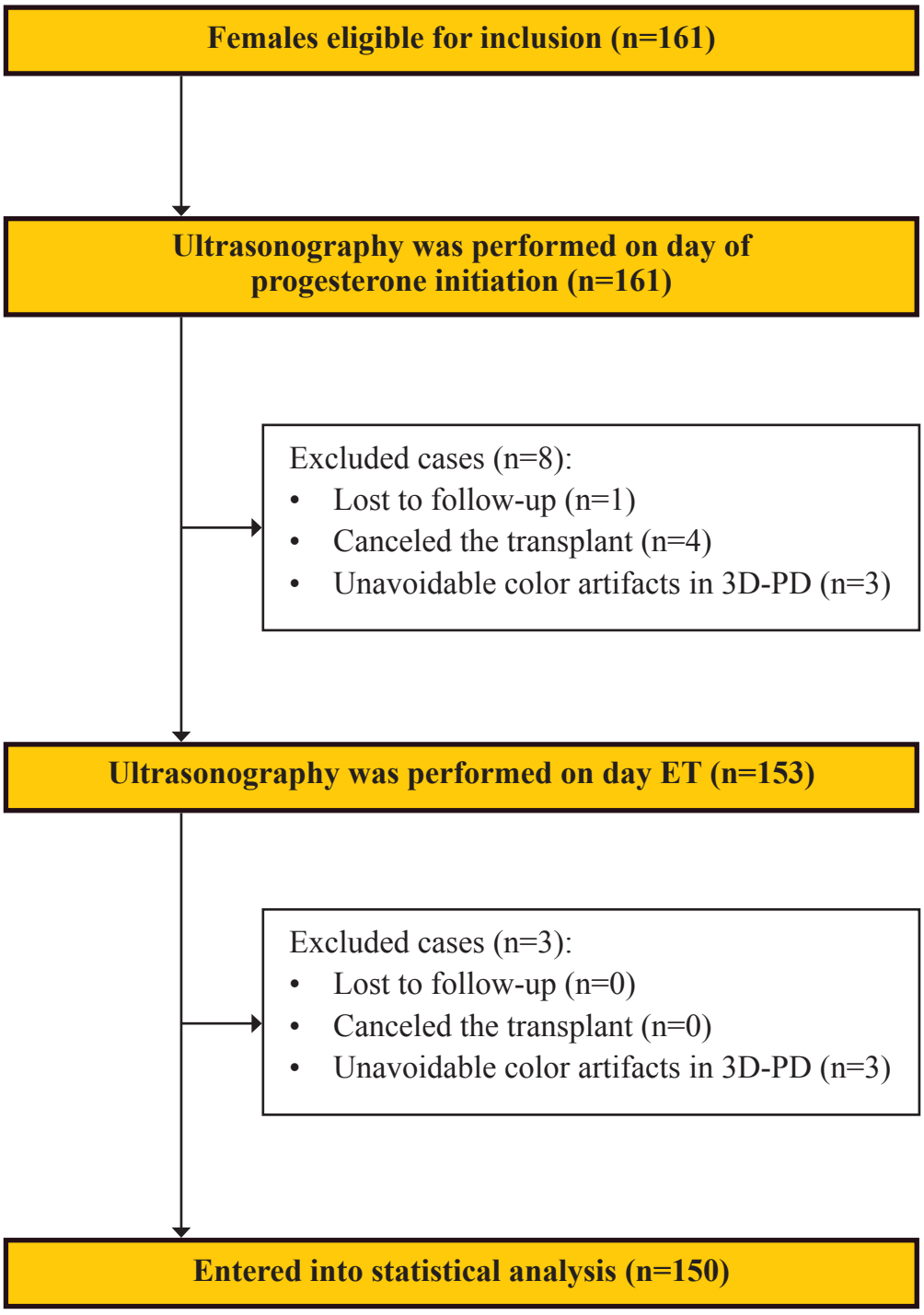

Figure.1. Flowchart of the study population

Table (1): Baseline demographic, clinical and ICSI cycle characteristics

Variables	Clinical pregnancy		P value
	Non Pregnant (N=48)	Pregnant (N=102)	
Age Mean ±SD	28.77±5.179	29.49±5.293	0.436
BMI Mean ±SD	25.22±3.05	24.86±2.88	0.477
Duration of infertility (years) Mean ±SD	3.88±1.77	4.16±1.84	0.378
AMH Mean ±SD	4.585±3.29	4.891±3.596	0.619
Basal FSH Mean ±SD	6.83±2.34	6.67±2.54	0.707
Basal LH Mean ±SD	10.77±5.12	11.53±5.07	0.397
Basal E2 Mean ±SD	36.79±3.73	35.82±3.63	0.134
Basal progesterone Mean ±SD	0.622±0.10	0.613±0.09	0.585
Duration of estrogen use Mean ±SD	12.77±1.89	12.81±1.52	0.882
Number of Embryos transferred Mean ±SD	1.9±0.515	2.00±0.613	0.310
G1 Embryos Mean ±SD	0.81±0.673	0.82±0.723	0.929
G2 Embryos Mean ±SD	1.06±0.783	1.16±0.853	0.518

Table (2): Different 3D endometrial parameters among pregnant and non-pregnant population

variables	Clinical pregnancy		P value
	Non Pregnant (N=48)	Pregnant (N=102)	
Endometrial thickness (day of progesterone) Mean ±SD	11.492±1.8353	11.336±1.8050	0.625
Endometrial thickness (ET day) Mean ±SD	11.498±2.1100	11.276±2.1269	0.550
Endometrial thickness difference	-.0067±.9923	.0605±1.3503	0.961
Endometrial volume (day of progesterone) Mean ±SD	4.8542±1.85610	4.5676±1.55195	0.324
Endometrial volume (day of ET) Mean ±SD	4.7065±1.83966	4.0006±1.70207	**0.022***
Endometrial volume difference	.14770±.5633	.56706±2.32506	**0.000***
VI (day of progesterone) Mean ±SD	2.1609±1.09386	2.0376±.99854	0.495
VI (day of ET) Mean ±SD	1.1490± .72997	1.3170±.82812	0.231
FI (day of progesterone) Mean ±SD	32.3700±7.27512	32.0771±6.57438	0.806
FI (day of ET)	31.1600±7.24133	30.5194±7.20617	0.613
VFI (day of progesterone) Mean ±SD	.6893±.35656	.6548±.35125	0.578
VFI (day of ET)	.3658±.26364	.3922±.25637	0.561

*significant, VI: vascular index, FI: flow index, VFI: vascular flow index

Table (3): Stepwise logistic analysis for the effect of 3D endometrial parameters on clinical pregnancy rate

	Coefficient	SE	Adjusted R^2	SEE	P value
Endometrial volume change percent	-0.10643	0.019986	0.009	0.351	**<0.0001****

**highly significant

Table (4): Validity of endometrial volume change percent in prediction of clinical pregnancy outcome among the studied groups:

Endometrial volume change percent	Cut-off point	AUC	Sensitivity (%)	Specificity (%)	95% Confidence interval (lower-upper)	Significance
	10.44	0.805	72.55%	77.08%	0.732 - 0.865	<0.0001**

AUC: Area under the ROC curve **highly significant

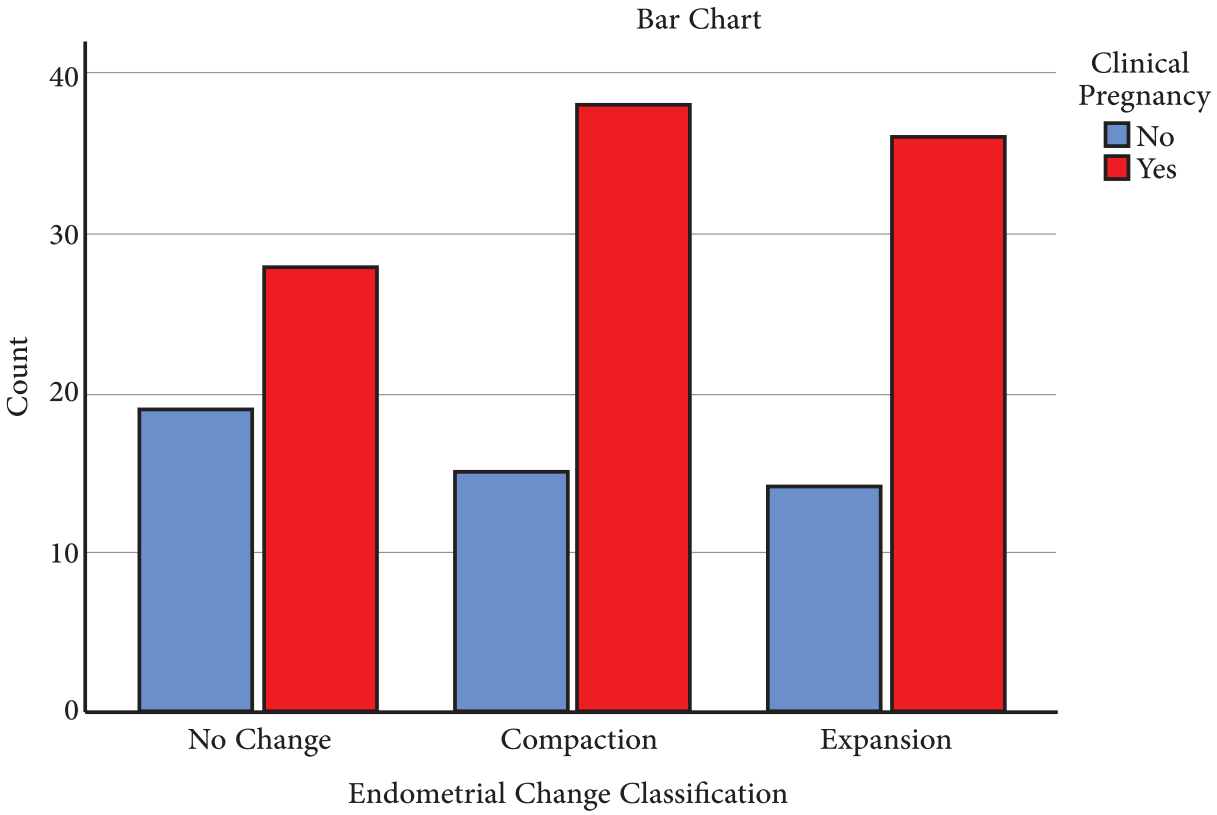

Figure 2. Clinical Pregnancy rate among endometrial thickness change groups (compaction, expansion, and stable groups)

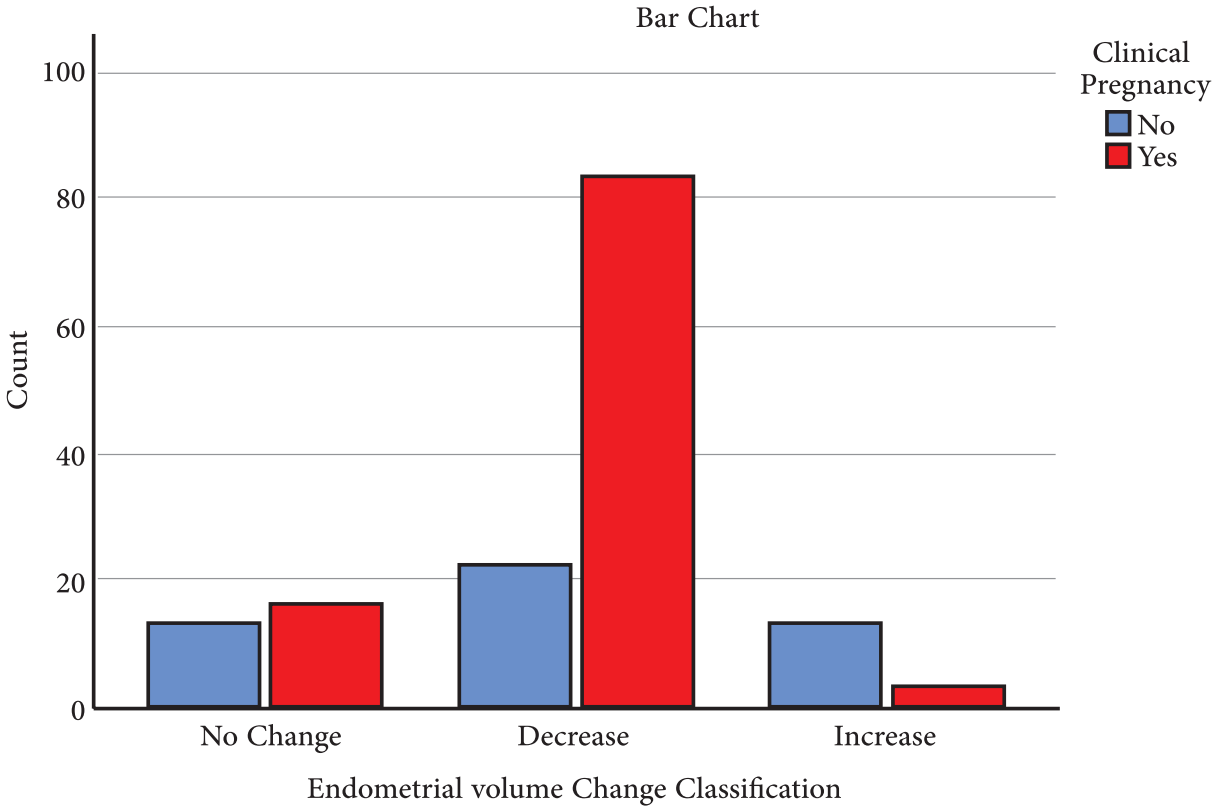

Figure 3.Clinical pregnancy rate among endometrial volume change groups (decrease, increase and stable groups)

Evaluation of Accuracy of Chromohysteroscopy as A Diagnostic Strategy for Perimenopausal Bleeding

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Abstract

Objective: To evaluate the role of endometrial dying with methylene blue during diagnostic hysteroscopy to detect new subtle histopathologies missed by the conventional hysteroscopy.

Methods: One hundred patients were included in the study complaining of perimenopausal bleeding with no diagnosed hysteroscopic endometrial abnormalities nor a general bleeding tendency. Endometrial dying was done by instillation of 5 ml of 1% methylene blue into the endometrial cavity after diagnostic hysteroscopy. According to the pattern of methylene blue staining, 60 patients showed focal dark staining with a background of light blue stained endometrium forming **(group I)**, the remaining 40 patients showed non focal diffuse blue staining forming **(group II)**. **Group I** was further subdivided into group A , B according to the age with group A (40-46 years) and group B (>46 years)..

Results: Chromohysteroscopy procedure led to the diagnosis of 48 more new endometrial histopathologies missed by conventional diagnostic hysteroscopy including 16 cases of atrophic endometritis, 29 cases of simple endometrial hyperplasia and 3 cases of atypical endometrial hyperplasia.The overall validity of the chromohysteroscopy procedure was calculated with a sensitivity of 93.2%, specificity of 87.8%, positive predictive value of 91.6 % and negative predictive value of 90%.

Conclusions: Chromohysteroscopy appears to improve the efficacy of hysteroscopy in abnormal uterine bleeding and observation of diffuse light blue staining without dark areas strongly suggests a normal endometrium free of endometritis.

Keywords: Chromohysteroscopy, Abnormal uterine bleeding, Endometrial biopsy, Methylene blue.

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Introduction

Malignant precursors of endometrial cancer such as complex endometrial hyperplasia, become more common during the perimenopausal period in comparison to younger age groups, and early diagnosis with endometrial biopsy should be done to exclude malignancy(1).

An endometrial biopsy is indicated in all patients with perimenopausal bleeding especially if the clinical history suggests a long term of unopposed estrogen exposure even if the endometrial thickness is normal (5 to 12mm)(2).

Traditionally, suspected endometrial pathologies have been investigated with blind biopsy techniques like dilatation and curettage, however, many focal lesions in the uterine cavity were missed with false negative rates between 3% and 7% (3).

Endometrial dying during conventional hysteroscopy using 1% methylene blue for enhancement and detection of subtle endometrial changes not detected by the naked eye during conventional hysteroscopy is a new technique named "Chromohysterosopy" named after the chromoendoscopy which is a widely used technique in gastrointestinal imaging(4).

Unlike the gastrointestinal mucosa, the endometrium is not an absorptive epithelium. Endometrium is not supposed to take any dye under normal circumstances. However, studies reported that endometrium stained by methylene blue except in the pre-ovulatory phase. The reason for endometrial staining is explained by apoptosis. They noted that structural damage of the cells during apoptosis would allow passage of the methylene blue dye into the cell(4).

Aim of Work

The aim of this work was to study the role of chromohysteroscopy as a new and simple modality for evaluation and diagnosis of endometrial pathology in cases of perimenopausal bleeding.

Patients and Methods

This observational cross section study was conducted at the Obstetrics and Gynecology department of Benha University Hospitals after receiving approval by the Research Ethical Committee of Benha Faculty of Medicine with the code RC 13-11-2023. Written Informed consent was obtained from all participants prior to commencing the study.

This study enrolled 100 patients who presented with complaint of perimenopausal bleeding and had the following criteria :

A- Inclusion criteria:

Women with perimenopausal bleeding, with no gross endometrial pathology that can be detected by the conventional diagnostic hysteroscopy.

B- Exclusion criteria:

- Patients with Contraindications of hysteroscopy, general causes of bleeding or not fit for general anaesthesia.
- Women on hormone replacement therapy.
- Patients with obvious ultrasound pathologies that could be the cause of the abnormal bleeding (like fibroid uterus).

All patients were subjected to:

1. Transvaginal sonography as a preliminary diagnostic tool to detect any uterine pathology and to measure the endometrial thickness
2. Conventional hysteroscopy followed by chromohy-steroscopy and the uterine cavity was visualized for staining pattern either focal or diffuse light blue staining, and the patients were divided into two groups according to the staining pattern: group I (with focal dark staining) and group II (with diffuse light staining)
3. In (group I), Two different biopsy spec-

imens were obtained from dark stained and light stained areas by hysteroscopic guided biopsy forceps while random curettage was done for (Group II) patients.

4. Different biopsy specimens were subjected to histopathologic examination using H&E.

Results

Comparison between group I & group II regarding age range showed a highly statistically significant increase in the appearance of focal dark stained areas in the age range above (>46yrs) while a higly significant elevation in diffuse light stained pattern was in the age range (40-46yrs) ($p<0.001$) (table 1).

Ultrasound evaluation resulted in exclusion of many cases, and from the included cases results of the histopathology of the endometrial samples were correlated with the results of the ultrasound calculated endometrial thickness, and showed a statistically non significant correlation between the endometrial thickness and the histopathology either in the group I or group II in the range of (5mm – 12mm) taken in the study where the mean endometrial thickness in group I was 8.7 mm and 9.02 in group II (table 2). Specimens from the dark stained areas in group I revealed that 18 patients (30%) had atrophic endometritis, 30 patients (50%) had simple endometrial hyperplasia, 4 patients (6.7%) showed complex endometrial hyperplasia without atypia , 3 patients(5%) had complex endometrial hyperplasia with atypia and 5 cases (8.3%) had a non neoplastic non inflammatory endometrium (table 2). Histopathological examination of specimens from the diffuse light stained areas in group I revealed 2 patients (3.3%) with atrophic endometritis, 3 pateints (5%) with simple endometrial hyperplasia, 4 patients (6.7%) with complex endometrial hyperplasia without atypia, and 51 patients (86%) with non neoplastic non inflammatory endometrium. (table 2). The total number of patients in group I who showed abnormal histopathological findings in focal dark stained areas were (55) , 48 of them (85.4%) were newly diagnosed by chromohystroscopy (table 2). Of the 40 patients included in group II, one case (2.5%) showed histopathological atrophic endometritis, 3 cases (7.5%) revealed simple endometrial hyperplasia & 36 cases (90%) showed non neoplastic non inflammatory endometrium (NNE) with a high statistically significant value (table 1).

There was a high statistically significant difference between the values of the mean age of the cases with atrophic endometritis (48.55±2.38), simple endometrial hyperplasia without atypia (47.54±2.26), complex endometrial hyperplasia without atypia (48.25±2.21), complex endometrial hyperplasia with atypia (49.0±2.82) and the non neoplastic non inflammatory endometrium (44.0±3.1) ($P<0.001$) (table 3) Of the 60 patients with focal dark stained areas 55 patients showed abnormal histopathological findings. This indicated a positive predicive value Of (91.6%) Of the 40 patients with diffuse light staining in chromohystroscopy, 36 showed normal findings .This indicated a negative predictive value of (90%).59 cases showed endometrial pathologies on microscopic examination. 55 of them showed focal dark stained areas with chromohysrtoscopy. This indicated that the sensitivity of chromohystroscopy was (93.2%) 41 patients had normal histopathologic examination, 36 of them showed no focal dark staining by chromohystroscopy. This indicated that specificity of chromohystroscopy was (87.8%) (table 4).

Table 1. Comparison between Group I and Group II according to age range, endometrial thickness measured by 2D transvaginal ultrasound and histopathological patterns in each group (values are given as n, % and mean ± SD)

	Group I (n = 60)	Group II (n = 40)	p
Age Range			
40 - 46 (A)	16 (26.7%)	31 (77.5%)	χ^2 <0.001*
> 46 (B)	44 (73.3%)	9 (22.5%)	
Endometrial Thickness (mm)			
Mean ± SD.	8.7±1.3	9.05±1.2	t 0.17
Histopathological patterns			
Atrophic endometritis	18 (30%)	1 (2.5%)	χ^2 <0.001*
Simple endometrial hyperplasia without atypia	30 (50%)	3 (7.5%)	
Complex endometrial hyperplasia without atypia	4 (6.7%)	0 (0%)	
Complex endometrial hyperplasia with atypia	3 (5%)	0 (0%)	
Non neoplastic non inflammatory endometrium	5 (8.3%)	36 (90%)	

χ^2: Chi square test t: Student t-test SD: Standard deviation mm: Millimeter
p: p value for comparing between the studied groups n: number
*: Statistically significant at p ≤ 0.05

Table 2. Frequency of different histopathological findings in group I regarding specimens retrieved from lightly stained and dark stained endometrium with statistical analysis of newly cases diagnosed by chromohysteroscopy

Group I (60 Patients had focal dark staining with a background of light stained endometrium)				
Histopathological patterns in relation to stained specimens	**Lightly stained specimens**	**Dark stained specimens**	**Newly diagnosed cases by chromohysteroscopy**	**p**
Detected Pathology (55/60) Atrophic endometritis (N = 18)	X (%) 2 (3.3%)	Y (%) 18 (30%)	(Y-X)/N 16/18 (89%)	χ^2 <0.001*
Simple endometrial hyperplasia without atypia (N = 30)	3 (5%)	30 (50%)	27/30 (96.7%)	χ^2 <0.001*
Complex endometrial hyperplasia without atypia (N = 4)	4 (6.7%)	4 (6.7%)	0/4 (0%)	------
Complex endometrial hyperplasia with atypia (N = 3)	0 (0%)	3 (5%)	3/3 (100%)	χ^2 <0.001*
NO Detected Pathology (5/60) Non neoplastic non inflammatory endometrium	51 (85%)	5 (8.3%)		

N: number of detected pattern per total group (60 cases)
X: number of detected pattern regarding specimen taken from lightly stained endometrium
Y: number of detected pattern regarding specimen taken from dark stained endometrium
p: p value for comparing between the studied groups χ^2: Chi square test
*: Statistically significant at p ≤ 0.05

Table 3. Statistical comparison between Mean values of age and frequency of histopathology in group I detected in darkly stained areas :

Histopathological pattern	Age (Mean± SD)	p
Atrophic endometritis (N = 18)	48.55± 2.38	$\chi^{2\,(FE)}$ <0.001*
Simple endometrial hyperplasia without atypia (N = 30)	47.54±2.26	
Complex endometrial hyperplasia without atypia (N = 4)	48.25±2.21	
Complex endometrial hyperplasia with atypia (N = 3)	49.0± 2.82	
Non neoplastic non inflammatory endometrium (N = 5)	44.0±3.1	

N: number of detected pattern per total group (60 cases) SD: Standard deviation FE: Fisher Exact
p: p value for comparing between the studied groups $\chi 2$: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (4): Validity of chromohysteroscopy in detection of abnormal endometrial pathologies missed by hystroscopy:

Validity	%
Sensitivity	93.2
Specificity	87.8
Positive predictive value (PPV)	91.6
Negative predictive value (NPV)	90

Discussion

With the advancement of hysteroscopy and outpatient sampling techniques, there is regression of D&C procedures, especially in developed countries, in the evaluation of abnormal uterine bleeding (5).

Indeed, the negative predictive value of hysteroscopy is >90%, when no structural abnormality is disclosed in a completely visualized uterine cavity and when the endometrium appears uniformly thin and homogeneous (6) theoretically, no further medical investigations should be necessary.

However, because of the frequent lack of hysteroscopic diagnosis in cases like endometritis, systematic endometrial sampling may be recommended for pathological examination, even though the hysteroscopic view is negative.

This study focused on the detection of the hidden endometrial abnormalities not detected by the conventional hysteroscopy causing the abnormal uterine bleeding in the perimenopausal age group through employing chromo-hysteroscopy as a new technique for hysteroscopic guided endometrial sampling.

The study included 100 patients complaining of perimenopausal bleeding, without any obvious medical or anatomical abnormalities explaining this bleeding evidenced by a meticulous history taking and a thorough general and local examination and transvaginal sonographic evaluation.

The patients were divided according to the pattern of staining into two groups: group I (with positive focal staining) and group II (without focal staining).

In this study , evaluation with transvaginal sonography was performed for all patients as a preliminary step to exclude cases with obvious pelvic pathology that could cause abnormal uterine bleeding and the final results of the endometrial samples were correlated with the results of the ultrasound calculated endometrial thickness, and showed a non significant correlation between the ultrasound calculated endometrial thickness and the final histopathology results. Other studies found transvaginal sonography as a very valuable sole diagnostic tool for the detection of the endometrial pathology in the perimenopausal age group; a difference which can be explained by the narrow cut off value for the endometrial thickness taken in our study (5-12 mm) and the exclusion of the cases with obvious pelvic pathologies like submucosal myoma and adenomyosis (7,8) .

The neglect of the three dimensional character of the endometrial cavity and the occasional propensity of the ultra -sonographic operator to obtain a limited number of two dimensional views and assume that this represents the entire endometrial cavity may also explain this non significant correlation ,Opening the way for the new three-dimensional ultrasound equipment to eliminate errors that may occur if the operator does not pay meticulous attention to mentally recreating three-dimensional anatomy (9).

Our result illustrates the observation that a diffuse light blue staining without focal dark areas strongly suggests a normal endometrium free of pathology; a finding which is in accordance with the other study that viewed dark staining in 19 of 24 cases of postmenopausal bleeding and discovered 3 new pathologies , two atrophic endometritis and one endometrial hyperplasia (10).

Another study by the same author on the role of chromohysteroscopy in the evaluation of the endometrium in recurrent IVF failure showed a sensitivity (69.2%), specificity (74%), positive predictive value (40.9%) and negative predictive value (90.2%) for detection of endometritis; where they suggested that removal of local endometrial defects may lead to replacement by healthy cells and receptivity is restored, eventually successful implantation is achieved.

However, this study had a fewer number of cases (only 63 patients) and a much different age range (women in the reproductive age range 24-37 years old) (11).

Our result showed that 55 histopathologies were dicovered in group I in the hysteroscopic guided specimens taken from the focal dark stained areas after being considered normal by the conventional hysteroscopy and included:

- 18 cases with Atrophic endometritis(over all 19 cases"19%" in both groups).
- 30 cases with Simple endometrial hyperplasia without atypia (overall 33 cases"33%") .
- 4 cases with Complex endometrial hyperplasia without atypia "4%" .
- 3 cases with Atypical endometrial hyperplasia"3%" .

And all the previous findings constituted abnormal findings, with no single case of overt malignant changes discovered.

5 cases in the group I with focal dark stained areas showed a non neoplastic non inflammatory endometrium "NNE"(overall 41 cases in both group I and group II "41%"). (including all the other histologic entities normaly found in endometrial specimens in this age group due the frequent occurrence of periods of anovulation like disordered proliferative endometrium (2)

In comparison to other a study done to evaluate the endometrial pathology in cases of perimenopausal bleeding where conventional D&C biopsy was performed for 748 cases with perimenopausal bleeding and showed 5 cases with atypical hyperplasia(0.7%) , 41 cases with simple hyperplasia

(6%) , 51 cases with atrophic endometritis (7%) ,550 cases with a non neoplastic non inflammatory pathology (74%) and only two cases with endometrial carcinoma (0.4%) (12) .

The findings which denoted a better detection rate of chromohysteroscopy for endometrial pathologies in the perimenopausal women with abnormal uterine bleeding especially for the diagnosis of atrophic endometritis and the precancerous lesion , atypical endometrial hyperplasia.

Regading group II, our result illustrates the Observation that a diffuse light blue staining without focal dark areas strongly suggests a normal endometrium free of pathology. Where most of the cases of group II (36 cases , 90%) showed a non neoplastic non inflammatory histology in their specimens; the results which are partially in agreement other study in terms of the validity of the test for diagnosis of endometritis. Because they studied a group of patients at younger age than our patients. Also the indications for hysteroscopy were different (dysfunctional uterine bleeding, failed intrauterine insemination, unexplained infertility and recurrent first trimester abortion).(13)

So chromohysteroscopy would be a very valuable simple office screening tool without the need for endometrial biopsy using only the simple office hysteroscopy followed by follow up by the same procedure after 6 months with a very high confidence.

The observation of micropolyposis was reported to be associated with endometritis in a study imploying fluid hysteroscopy aiming at the detection of floating endometrial micropolyps with the high resolution hysteroscopic image, however in this study about half of the cases were misdiagnosed (with a sensitivity of about 57%) when compared to the final diagnosis by the histopathology (14).

In current study , when endometrial dying was employed instead of observation of micropolyposis, with the diagnosis of 55 histopathologies in a total no. of 100 cases with 48 (87.3%) new histopathologies diagnosed only in the focal darkly stained areas with a special increased sensitivity for the diagnosis of atrophic endometritis (16 newly diagnosed cases) and simple endometrial hyperplasia (29 new cases) missed by the conventional hysteroscopic view, and three cases of atypical endometrial hyperplasia diagnosed only by chromohysteroscopy with a very high significance measured by Mc Nemar's test with a Sensitivity of (93.2%) , specificity (87.8 %), +ve predictive value (91.6%) , -ve predictive value (90%).

Endometrial hyperplasia is a histological diagnosis characterized by the proliferation of the endometrial glands resulting in a greater gland-to-stroma ratio than observed in normal endometrium (15) and when pre-operative endometrial sampling shows atypical complex hyperplasia, the pathologist frequently has to sample the entire endometrium to rule out a focus of endometrial carcinoma. This means that over 10 cassettes of formalin-fixed, paraffin-embedded tissue must be examined and the chromohysteroscopy may allow for more accurate sampling with no need for a vast number of formalin-fixed, paraffin-embedded tissue. (16).

Recommendations

In this study, Endometrial dying was able to detect new subtle endometrial pathology which was missed by conventional hysteroscopy especially when target biopsy taken from dark-stained endometrium; so we recommend that application of chromohysteroscopy would improve the efficacy and reliability of endometrial guided biopsy in cases of abnormal uterine bleeding.

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Intraperitoneal Gas Drain to Reduce Postsurgical Shoulder Tip Pain in Gynecological Laparoscopy

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Running Title

Intraperitoneal Drain After Laparoscopy

Abstract

Background : Operative gynecologic laparoscopy has become the preferred method for treating benign gynecologic diseases due to its minimally invasive nature and faster recovery compared to laparotomy. However, post-laparoscopic shoulder pain (PLSP) is a common complaint following laparoscopic surgeries, affecting patients' satisfaction and recovery. Various methods have been proposed to alleviate PLSP, but consensus on their effectiveness remains elusive.

Aim of the Work : This randomized controlled clinical trial aims to investigate the effect of gas drainage by intraperitoneal drain on shoulder pain in women after laparoscopic surgery in comparison to no drain use.

Patients and Methods: A randomized controlled clinical trial involving 120 female patients undergoing laparoscopic surgery was conducted to investigate the effect of intraperitoneal drainage on postoperative shoulder pain. The patients were divided into two groups: the study group (n=60) with intraperitoneal drain placement, and the control group (n=60) with routine technique and no drain use. Visual Analog Scale (VAS) scores were used to assess shoulder and abdominal pain at different postoperative time points.

Results: The study demonstrated that intraperitoneal drainage significantly reduced postoperative shoulder pain in the first 12 hours after surgery compared to the control group ($p < 0.001$ at 3 and 6 hours, $p = 0.038$ at 12 hours). However, no significant difference in shoulder pain was observed between the two groups at 24 hours post-surgery ($p = 0.451$). The need for postoperative analgesia was also lower in the drainage group ($p < 0.001$). These findings align with previous studies suggesting the efficacy of drainage in reducing shoulder pain after laparoscopic surgery.

Conclusion: This study demonstrates the effectiveness of intraperitoneal drainage in reducing post-laparoscop-

ic shoulder pain during the first 24 hours after surgery, consequently reducing the need for postoperative analgesics. These findings support the outcomes of previous investigations, indicating that drain placement may be a valuable strategy to alleviate postoperative shoulder pain in women undergoing gynecologic laparoscopy.

Keywords: Laparoscopy, postoperative pain, shoulder pain, intraperitoneal drainage.

INTRODUCTION

Pain is the most common complaint among patients and a source of concern for medical personnel, and pain management is a significant part of patient satisfaction[1].

With the improvement in medical research, several procedures for treating patients have become accessible, including laparoscopy, which is becoming the gold standard approach for many conditions. [2]In comparison to open surgeries, Laparoscopy caries less complications, better recovery and shorter hospital stay. [3]

It is expected that 30–80% of cases experience shoulder pain following laparoscopic surgery, especially in the first 24 hours [4]. The residual gas causes stretching of the post-distended diaphragm and peritoneum after prolonged surgery in the abdominal cavity, causes shoulder pain [5]. There are many recommended pharmacological agents that has been used to reduce post laparoscopy pain, but due to their side effects, researchers have looked at non pharmacological options. [6]

In some laparoscopic procedures, such as cholecystectomy, the use of an intraperitoneal drain showed conflicting results [7].

For the treatment of shoulder pain after laparoscopy, therapies such as subcutaneous anesthetic injections, regular saline injections into the abdominal cavity, and lowering the pressure of CO2 gas flow are commonly used [8].

Since an intraperitoneal drain is a medical device for emptying and suction, it may be useful for removing gases from the abdominal cavity and eventually reducing post laparoscopy pain. [9].

AIM OF THE WORK

This randomized controlled clinical trial aimed to investigate the effect of gas drainage by intraperitoneal drain on shoulder pain in women after laparoscopic surgery in comparison to no drain use.

PATIENTS AND METHODS

This prospective randomized controlled clinical trial was conducted at operative theatres, Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University Hospitals from January until July 2023.

Study population: Female patients attending Ain Shams University Maternity Hospital for laparoscopic surgery with the following criteria:

Inclusion criteria: Women > 18 years old, women with benign gynecological conditions or indications for diagnostic laparoscopic procedures and laparoscopic duration: minimum 15 minutes and maximum 60 minutes.

Exclusion criteria: Women with chronic abdominal, pelvic and shoulder pain or trauma, women whose laparoscopic surgery changes to laparotomy and women who are not willing to participate in the study or unable to sign consent.

Sampling Method "randomization": Systematic random sampling and women fulfilled the inclusion criteria were randomly assigned to either group. One Hundred Twenty opaque envelopes were numbered serially and, in each envelope, the corresponding letter, which denoted the allocated group, was put according to randomization table. Then all envelopes were closed and put in one box. Randomization was done using computer generated randomization sheet using MedCalc © version 13.

Sample size: The study was conducted on 120 women; they were subdivided into 2 groups. The required sample size calculated based on the following equation: n = required sample size per group = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail) n= 0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%).

Sample size justification: Using PASS 11 program for sample size calculation, setting confidence level at 95%, margin of error at ± 0.1 and by reviewing results from previous study 9 showed the rate of severe pain among patients underwent female laparoscopic surgery with intraperitoneal drain versus without drain (control) were (2.6% vs. 15.8% respectively); based on that the required sample size will be at least 57 patients undergoing laparoscopic surgery in each group to be sufficient to achieve study objective.

Ethical considerations:

Patient information and informed consent: before being enrolled into the study, all the study procedure was discussed with the patients and an informed consent was obtained from whom who approved to parti consented to participate after the nature, scope and possible consequences of the clinical study had been explained in a form understandable to her.

Confidentiality: only the patient initials were recorded in the case report form, and when the patient's name appeared on any other document, it was kept in a secure place by the investigators. The investigators maintained a personal patient identification list (patient initials with the corresponding patient names) to enable record to be identified.

Protocol approval: before the beginning of the study and any accordance with the local regulation followed, the protocol and all the corresponding documents were declared for ethical and research approval by the council of Obstetrics and Gynecology Department, Ain Shams University.

Concerning safety and efficacy: Complications of laparoscopy.

Study interventions and procedures:

The following data were collected: Women age, BMI, indications of laparoscopy, previous surgical and medical history.

Procedure details including: Number of ports entered the abdomen, amount of gas used during the procedure, findings on entry including: any surgical intervention done during procedure (adhesiolysis, cystectomy, dye test, etc …), any complications during the procedure, time of surgery and postoperative analgesia requirement (according to VAS)

Anesthesia was established after intubation with number 7 or 7.5 tube using anesthetic drugs (1% isoflurane and 50% N2O with 50% oxygen).

Laparoscopic surgery was performed using carbon dioxide gas and three ports will be inserted as follows: A 10 mm port under the umbilical cord to enter the telescope and two 5 mm ports through the outer edge of the rectus muscle to enter the surgical instrument. CO2 gas was entered into the peritoneum with an initial low flow then high flow will be allowed. CO2 gas blower unit was adjusted at gas pressure between 12 and 16 mm Hg during the operation. The same procedure was performed for all the participants of this research. Study population was divided into two equal groups:

Group I (study group): Consisted of 60 women to whom intraperitoneal drain was placed.

Group II (control group): Consisted of 60 women to whom routine technique without drain was used.

In the study group, before closure of the skin, intraperitoneal passive drain (Nelaton catheter size 14) was inserted through any of the side ports without extra incision and placed in RLQ (Right lower quadrant) in pelvis at surgical point through the outer edge of the

rectus. It was entered into the surgical port and then the skin was sutured with 3/0 vicryl thread. In the control group, routine laparoscopic surgical technique without drain was performed. In both groups, the pain in abdomen and shoulder was recorded at 3, 6, 12, and 24 h after the surgery using Visual Analogue Scale (VAS) of pain. As per hospital protocol, analgesia was given at 12 hrs. interval. If extra analgesia was needed a dose of Diclofenac sodium suppositories (Voltaren 100mg, NOVARTIS PHARMACEUTICALS) was given at least 6 hrs. Apart from the previous analgesia if the patient's pain score = or > 4.

Study outcomes:

Primary outcome: The severity of shoulder pain in study groups at 3, 6, 12, and 24 h after surgery using Visual Analogue Scale (VAS) of pain. Based on the distribution of pain VAS scores in postsurgical patients who described their postoperative pain intensity as none, mild, moderate, or severe the following cut points on the pain VAS has been recommended: 0 = No pain. 10 = Max. pain. 0 – 3 = mild pain. >4 – 7 = moderate pain. >7 – 10 = severe pain. [10].

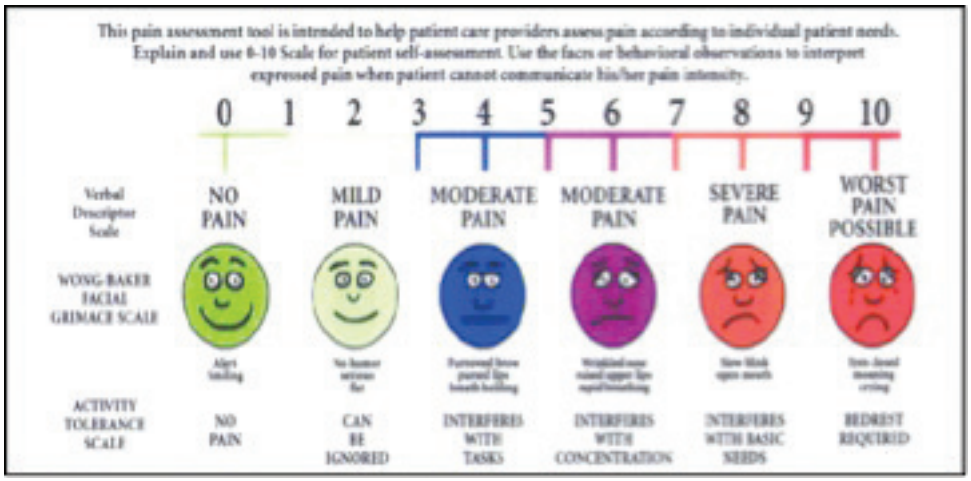

Figure 1: Visual analog scale with corresponding Wong-Baker Faces Scale (WBS) [11]

Secondary outcomes: Severity of abdominal pain, the number of Doses of analgesia, postsurgical adverse events as fever, nausea, vomiting and wound complications, hospital stay and patient satisfaction.

Statistical analysis: Data were collected, coded, revised, and entered into the Statistical Package for Social Science (Rstudio) version 2.3.2. The data were presented as numbers and percentages for the qualitative data, mean, standard deviations, and ranges for the quantitative data with parametric distribution, and median with interquartile range (IQR) for the quantitative data with the non-parametric distribution. **The Shapiro test** was used to verify the normality of the distribution. **The chi-square** test was used in the comparison between two groups with qualitative data and **Fisher exact test** was used instead of the Chi-square test when the expected count in any cell was found less than 5.

Independent t-test was used in the comparison between two groups with quantitative data and parametric distribution and **Wilcoxon Mann-Whitney test** was used in the comparison between two groups with quantitative data and non-parametric distribution.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: $P > 0.05$: Non-significant (NS), $P < 0.05$: Significant (S) and $P < 0.01$: Highly significant (HS).

RESULTS

Baseline characteristics:

This study was carried out on one hundred and twenty patients (120 patients) divided into two groups. Group (1) consist of 60 patients and Group (2) consist of 60 patients. The comparison between the two groups including age, BMI, parity, mode of previous delivery, past medical and surgical history showed no statistically significant difference as shown in Table (1). Also the indication of laparoscopy was statistically insignificant if compared between the two groups.

Procedure details:

As regard of duration of procedure, Table 2 showed equivalent results regarding the time of the laparoscopy between the two groups and the number of ports inserted. The procedure done were similar statistically between the two groups except for the number of cystectomies which was higher with statistically significant difference in group 1 compared to group 2. (Table (2)

VAS score of shoulder pain:

For the VAS score of shoulder pain at 3 hours, 6 hours, 12 hours, the results showed a significant statistical difference between the groups regarding perception of pain with the study group having less pain if compared to the control group.

For the VAS score of shoulder pain at 24 hours, in Group (1) and Group (2), the scores ranged from 0 to 2 and 0 to 3 respectively. The mean & SD for Group (1) and Group (2) were 0.47±0.70 and 0.63±0.90, respectively, so both groups showed equivalent result with no statistically significant difference between them regarding pain perception as shown in Table (3).

VAS score of abdominal pain:

Similar to the shoulder tip pain perception, the VAS score of abdominal pain at 3, 6 and 12 hours, showed statistically significant different scores in study group if compared to the control group with the lower pain scores in the study group while there was no significant difference at 24 hours after the laparoscopy. Table (4)

Need for extra analgesia:

According to the need for extra analgesia, only 16 patients (26.7%) needed extra analgesia in the group (1). However, 40 patients (66.7%) needed extra analgesia in the group (2). There was a highly significant difference in the need for extra analgesia between the two studied groups at ($p<0.001$) as shown in Table (5).

DISCUSSION

Operative gynecologic laparoscopy is becoming the primary approach for the treatment of benign gynecologic diseases, as it is a less invasive procedure, helps shorten the length of hospitalization, and facilitates recovery earlier than laparotomy [12-13]

Most complications of laparoscopic procedures occur during abdominal access or port placement, while other complications arise during abdominal insufflations, tissue dissection, and homeostasis 14. However, post-laparoscopic shoulder pain (PLSP) has been the most common complaint that often occurs following laparoscopic surgeries and has an important impact on patients' satisfaction 15. It was reported that the incidence of PLSP ranges from 35–80%, and the intensity varies from mild to severe [16-17]

Although the exact mechanism of PLSP remains unclear, some studies have suggested that it is caused by the trapping of carbon dioxide (CO2) between the liver and the right diaphragm and subsequent conversion into carbonic acid, which irritates the diaphragm and subsequently generates referred shoulder pain (C4 dermatomal) [18-19]

To reduce the post-laparoscopic shoulder pain, several methods have been suggested including the use of a peritoneal gas drain in the first 4–6 hours following laparoscopy, intraperito-

neal local anesthesia, pulmonary recruitment maneuver, intraperitoneal saline infusion, gasless laparoscopy and reduction in insufflation pressure. It has been suggested that these methods reduce post-operative shoulder pain by decreasing the volume of residual intraperitoneal gas, but there is no consensus among researchers regarding the effectiveness of the above-mentioned methods [20-23]

Herein, this study aimed to investigate the effect of drainage by intraperitoneal drain on shoulder pain in women after laparoscopic surgery in comparison to no drain use. The results as described above showed the reduced VAS scores and need of extra analgesia in the post operative first 12 hours and similar with the control group by 24 hours.

These findings indicate the effectiveness of drainage by an intraperitoneal drain in reducing postoperative pain in the first 24 hours of gynecological laparoscopy to the extent that reduces the need for postoperative analgesics.

These findings support the results of previous studies. Haghoo et al investigated the drainage for peritoneal suction to reduce shoulder pain caused by gynecological laparoscopy. They found at 12 h and 24 h after surgery, the VAS score for shoulder pain was statistically lower in the group with drainage ($P < 0.001$ for both), but not after 48 hours post-surgery ($P = 0.806$). Also, significantly higher postoperative demand for analgesics was observed in the control group ($P < 0.001$). The authors concluded that gas drainage may be useful for preventing postoperative shoulder pain among patients undergoing gynecological laparoscopic surgery and could decrease the need for pain medication [24]

Chauhan and Vaishnav (2016)25, reported that drain insertion increases the duration of procedure and hospitalization, which may be due to the presence of the patients for post-surgical follow-up; meanwhile, the type of drain used was not mentioned in their study.

Likewise, Tharanon and Khampitak showed that the postoperative intra-abdominal tube drain could be an effective method for improving postoperative pain at nearly all parameters, including decreasing the need for postoperative morphine in long-time operations (> 2 hours)[16]

Hosseinzadeh et al. also agreed with this study findings. They found the severity of shoulder pain was significant between drain and control groups 3, 6, 12, and 24 h after surgery ($p < 0.001$). Consumption of diclofenac after operation was higher in the control group ($p < 0.001$). They suggested the use of a drain in female laparoscopic surgery is beneficial for reducing subsequent shoulder pain [9]

Also, a meta-analysis by Kaloo et al. revealed an association between the intraperitoneal drain and a reduction in the incidence and severity of shoulder pain when compared with no intraperitoneal drain at all time points assessed postoperatively at 3-4 hours, 12 hours, 24 hours, and 48 hours [26]

On the other side, an earlier randomized trial by Abbott et al. studied the effect of drainage use on postoperative shoulder pain after minor gynecological laparoscopic surgery and found that, although drainage use did not change the severity of shoulder pain, its use decreased the incidence of pain. However, the study showed that simply using an analgesic was more cost-effective compared with drainage use and did not recommend routine use of drains to prevent postoperative shoulder pain [27]

Again, contrary to this study results, a meta-analysis conducted by Craciunas et al. 21did not support the routine use of a peritoneal gas drain following gynecological laparoscopy because of very little evidence of an overall benefit from this approach, and in addition, no association with a reduction in the requirement of analgesia and anti-emetics for shoulder pain and total pain when compared to no use of peritoneal gas drain

group. However, the authors recommended future studies to minimize the bias resulting from operating time and the use of the analgesic dosage as an objective measure for pain evaluation.

This was followed by a study that found the VAS scale was similar between the drainage and non-drainage groups ($p = 0.376$ and $p = 0.847$, respectively). They explained this by that drainage use may cause discomfort because of irritation, tissue damage, adhesion, obstruction, or entanglement, suggesting that the increase in postoperative abdominal pain may be because of the presence of the drain itself [28]. Asgari et al., 2018 [29] , also reported no effect of the drain in reducing post laparoscopy pain although they didn't evaluate the first 12 hours post-surgery.

Reduction of pain after pneumoperitoneum was investigated by several studies and different modalities were used. Bogani and colleagues [30]compared low-pressure (8 mm Hg) versus standard-pressure (12 mm Hg) pneumoperitoneum and found that while abdominal pain was similar between groups, the incidence of shoulder tip pain in the early postoperative period was 36% in the standard and 5% in the low-pressure group. Madsen and colleagues demonstrated less shoulder tip pain with lower inflation pressure [31]. When carbon dioxide (CO2) was humidified and heated, postoperative shoulder tip pain scores, but not abdominal pain scores, were lower than when using control gases[32] Lastly, elimination of CO2 with an open umbilical trocar decreased postoperative pain scores, but additional trocar site infiltration did not decrease pain scores or opioid consumption further [33]

Researchers have looked at the effect of the gas drain in different laparoscopic procedures with promising results 34-36. Further studies on larger scales and on lengthy procedures are recommended to validate the use of this simple, available technique to improve patients' satisfaction after surgical laparoscopic procedures.

CONCLUSION

The use of an intraperitoneal gas drain could significantly improve postoperative shoulder and abdominal pain in the first 24 hours resulting from gynecologic laparoscopic surgery. In addition, it reduces the need for postoperative analgesics. It is a cheap option with minimal side effect profile which might increase patient post operative satisfaction.

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Table (1): The Comparison between the two studied groups according to baseline characteristics

		Group (1) (n=60)	Group (2) (n=60)	p-value
Age in years	**Min.- Max.**	19 - 44 years	19 – 41 years	0.962
	Median (IQR)	29.5 (24.0 - 35.0)	30.0 (24.0 - 34.2)	
BMI (Kg/m^2)	**Min. – Max.**	20.8 – 39.5	21.5 – 38	0.362
	Median (IQR)	27.7 (24.7 - 31.0)	26.5 (24.5 - 29.0)	
Parity	**Nulliparous**	36 (60.0%)	37 (61.7%)	0.638
	Multipara	24 (40.0%)	23 (38.3%)	
Indication	**Ovarian cyst**	13 (21.7%)	3 (5.0%)	0.080
	1ry or 2ry infertility	34 (56.7%)	43 (71.7%)	
	Hydosalpinx	7 (11.7%)	10 (16.7%)	
	Ectopic	1 (1.7%)	1 (1.7%)	
	Missed IUCD	5 (8.3%)	3 (5.0%)	
Medical history	**Free**	47 (78.3%)	48 (80.0%)	0.582
	Rheumatic arthritis	1 (1.7%)	1 (1.7%)	
	Rheumatic fever	0 (0.0%)	1 (1.7%)	
	Bronchial asthma	3 (5.0%)	1 (1.7%)	
	Endometriosis	0 (0.0%)	1 (1.7%)	
	TB endometritis	0 (0.0%)	1 (1.7%)	
	FMF on colchicine	0 (0.0%)	1 (1.7%)	
	Hypothyroid on L-thyroxine 50	1 (1.7%)	2 (3.3%)	
	Diabetes	3 (5.0%)	3 (5.0%)	
	HCV	0 (0.0%)	1 (1.7%)	
	Epilepsy	2 (3.3%)	0 (0.0%)	
	HTN	1 (1.7%)	0 (0.0%)	
	TB salpinx	1 (1.7%)	0 (0.0%)	
	Osteosarcoma	1 (1.7%)	0 (0.0%)	
Surgical history	**Abdominal pelvic surgery**	35 (58.3%)	41 (67.8%)	0.272
	No surgery	21 (35.0%)	13 (22.0%)	
	Other non-abdominal surgeries	4 (6.7%)	6 (10.2%)	

Table (2): The Association between the two studied groups according to the procedure details

		Group (1) (n=60)	Group (2) (n=60)	p-value
Duration of procedure	**Min. – Max.**	20-60	20-60	0.094
	Mean±SD	39.83±11.75	36.42±10.38	
Number of ports	**2**	17 (28.3%)	21 (35.0%)	0.432
	3	43 (71.7%)	39 (65.0%)	
Procedure done	**MB +ve tubal patency test**	31 (51.7%)	39 (65.0%)	0.141
	Adhysiolysis	18 (30.0%)	20 (33.3%)	0.699
	Cystectomy	18 (30.0%)	3 (5.0%)	<0.001**
	Tubal disconnection	7 (11.7%)	9 (15.0%)	0.458
	Removal of IUCD	5 (8.3%)	3 (5.0%)	0.470
	Ovarian drilling	2 (3.3%)	5 (8.3%)	0.243
	Salpingectomy	2 (3.3%)	1 (1.7%)	0.576
	Detorsion and plication of ovarian ligament	0 (0.0%)	1 (1.7%)	0.313

Table (3): The Comparison between the two studied groups according to the VAS score of shoulder pain:

		Group (1) (n=60)	Group (2) (n=60)	p-value
VAS score 3 hours	**Min. – Max.**	0-3	0-5	<0.001*
	Mean±SD	2.02±0.77	2.58±0.81	
	Mild (0-3)	60 (100.0%)	56 (93.3%)	
	Moderate to severe (≥4)	0 (0.0%)	4 (6.7%)	
VAS score 6 hours	**Min. – Max.**	0-5	0-6	<0.001*
	Mean±SD	2.33±1.59	3.42±1.68	
	Mild (0-3)	45 (75.0%)	23 (38.3%)	
	Moderate to severe (≥4)	15 (25.0%)	37 (61.7%)	
VAS score 12 hours	**Min. – Max.**	0-3	0-4	0.045*
	Mean±SD	1.58±1.23	2.02±1.21	
	Mild (0-3)	60 (100.0%)	58 (96.7%)	
	Moderate to severe (≥4)	0 (0.0%)	2 (3.3%)	
	Min. – Max.	0-2	0-3	0.260
	Mean ±SD	0.47±0.70	0.63±0.90	
	Mild (0-3)	60 (100.0%)	60 (100.0%)	
	Moderate to severe (≥4)	0 (0.0%)	0 (0.0%)	

Table (4): The Comparison between the two studied groups according to the VAS score of abdominal pain

		Group (1) (n=60)	Group (2) (n=60)	p-value
VAS score 3 hours	**Min. – Max.**	2-3	1-5	<0.001*
	Mean±SD	2.27±0.45	2.85±0.94	
	Mild (0-3)	60 (100.0%)	50 (83.3%)	
	Moderate to severe (≥4)	0 (0.0%)	10 (16.7%)	
VAS score 6 hours	**Min. – Max.**	1-6	2-6	<0.001*
	Mean±SD	3.40±1.11	4.12±1.15	
	Mild (0-3)	45 (75.0%)	22 (36.7%)	
	Moderate to severe (≥4)	15 (25.0%)	38 (63.3%)	
VAS score 12 hours	**Min. – Max.**	1-4	2-4	0.046*
	Mean±SD	2.73±0.63	2.55±0.59	
	Mild (0-3)	57 (95.0%)	57 (95.0%)	
	Moderate to severe (≥4)	3 (5.0%)	3 (5.0%)	
VAS score 24 hours	**Min. – Max.**	1-3	0-3	0.298
	Mean±SD	1.55±0.57	1.67±0.66	
	Mild (0-3)	60 (100.0%)	60 (100.0%)	
	Moderate to severe (≥4)	0 (0.0%)	0 (0.0%)	

Table (5): The Association between the two studied groups according to Need for extra Analgesia:

Need for extra analgesia	Group (1) (n=60)	Group (2) (n=60)	p-value
No	44 (73.3%)	20 (33.3%)	**<0.001***
Yes	16 (26.7%)	40 (66.7%)	

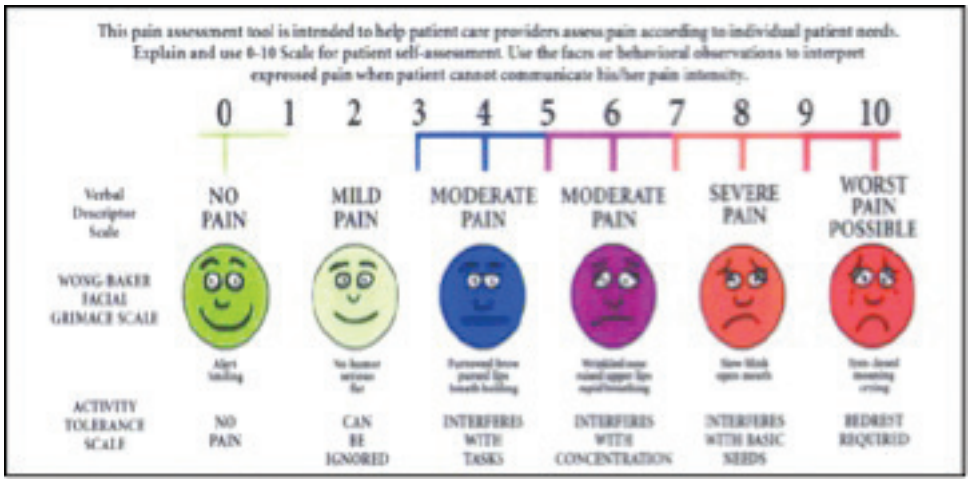

Figure 1: Visual analog scale with corresponding Wong-Baker Faces Scale (WBS) [11]

Pain Relief for Office Hysteroscopy: A Randomized Controlled Trial Comparing a Transcutaneous Electric Nerve Stimulation to Placebo

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Running title

Pain Relief for Office Hysteroscopy: A Randomized Controlled Trial Comparing a Transcutaneous Electric Nerve Stimulation to Placebo

Abstract

Background: More research is needed to determine the efficacy of transcutaneous electrical nerve stimulation (TENS) as a non-invasive pain control technique during office-based hysteroscopy.

Objective: To assess the analgesic effects of transcutaneous electrical nerve stimulation (TENS) in office hysteroscopy procedures, as well as patient satisfaction with this intervention.

Patients and Methods: The study included 120 female volunteers who underwent office hysteroscopy and were divided into two groups: TENS group (60 individuals) who got active TENS via a healthtronic alpha wave instrument, and the Placebo group (60 participants) who received placebo TENS.

Results: The study declared that both TENS and placebo groups reported equal degrees of pain during hysteroscopy, with no significant changes in pain severity pain ($p = 0.11$) or satisfaction ratings. The VAS score linked positively with age and parity, but adversely with height and the Likert verbal scale.

Conclusion: The TENS device does not significantly alleviate the pain associated with office hysteroscopy.

Keywords: Hysteroscopy, TENS, Pain.

Introduction:

Hysteroscopy is a reliable and efficient method for treating intracavitary uterine problems, including thick endometrium caused by postmenopausal bleeding, suspected endometrial cancer, Abnormal uterine bleeding, infertility, or recurrent pregnancy loss. It is a safe and convenient procedure that requires minimal invasiveness. [1]

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The hysteroscopy procedure involves the insertion of a device into the uterus through the cervix. It has the potential to be either rigid or flexible. Distention media, commonly saline, is employed to facilitate the enlargement of the uterine cavity. Subsequently, the hysteroscope is employed to visualize the endometrium, and if necessary, endometrial biopsies may be. performed. [1]

While the majority of women exhibit a high level of tolerance, with a successful completion rate ranging from 77% to 97.2%, the presence of pain can impede the successful completion of many subsequent hysteroscopies due to the resulting inconvenience. [2]

The efficacy of medications, paracervical or intracervical blockers, and topical analgesia in reducing pain intensity has been uneven, with no single method demonstrating the highest level of effectiveness. [3] TENS, or transcutaneous electrical nerve stimulation, is a relatively unexplored method for mitigating pain during office-based hysteroscopy. TENS is a cost-effective, non-intrusive, and secure therapy option, with few negative effects or drug interactions. The application of electrical currents to the skin is employed as a means of pain relief, bypassing the necessity for pharmaceutical interventions via both peripheral and central pathways. [4]

TENS machines administer low levels of electrical current to vibration receptors in order to stimulate them and mitigate the transmission of painful stimuli to the brain. The repeated administration of transcutaneous electrical nerve stimulation (TENS) units to a specific region elicits the secretion of endorphins, which inherently alleviates pain. Due to this rationale, they possess the capability to address both acute and chronic pain.(6)Multiple studies have demonstrated the efficacy and adequacy of transcutaneous electrical nerve stimulation (TENS) in the management of gynecological and obstetric pain, encompassing the treatment of labor pain and postpartum pain. [7], [8] Hence, it is crucial to investigate the efficacy of TENS in managing pain associated with office hysteroscopy in the study.

Patients and Methods

This study used a double-blind randomized controlled design and included 120 female patients who underwent office hysteroscopy at Ain Shams Maternity University Hospital's Early Cancer Detection & Gynecology Unit. The study ran from March to July 2019. The study was formally filed on the ClinicalTrials.gov website with the unique identifier NCT04229576.

The study included female patients aged 21 to 74 with leiomyoma or endometrial polyps, postmenopausal hemorrhage with thick endometrial lining, menstrual disturbance, or suspected uterine deformity. Women with unexplained vaginal hemorrhage, neurological impairments, opioid or psychotropic drug use, either preprocedural or chronic, prior transcutaneous electrical nerve stimulation (TENS), automatically implanted pacemakers, or cardiac defibrillators were excluded from this trial.

The patients were randomly assigned to two equal-sized groups, as indicated below. Group 1 (the study group) included 60 patients who received Active TENS during office hysteroscopy. In contrast, group 2, sometimes known as the control group, included 60 patients who got Placebo TENS. Participants in the Placebo TENS group were attached to the TENS device in the same way as those in the Active TENS group. However, during office hysteroscopy, the equipment produced the active indication light and sound but provided no electrical stimulation. All patients received Upon arrival, a thorough clinical examination was performed, and a precise medical history was obtained (Figure 1).

The allocation concealing strategy employs the use of successive numbers on opaque, sealed envelopes. These envelopes are assigned the letters "A" or "B" depending on

a computer-generated sequence. Each envelope includes a letter corresponding to a number from the randomization list. The note enclosed in the mail determined the allocation of participating ladies to each group.

The TENS intervention is also known as the active TENS instrument. The Healthtronic Alpha Wave comes with four wide pads, two channels, and two leads. The pulse width is 200 μs, and the frequency ranges from 40 to 100 Hz. The MB-1004 model had a tunable high-frequency range of 80 to 100 Hz with a pulse duration of 400 microseconds. The TENS treatment began 5 minutes before the hysteroscopy surgery and continued throughout the operation. Two sets of self-adhesive electrodes (circular pads) were placed parallel to the spinal cord at T10-L1 and S2-S4 levels.

The primary outcome of the office hysteroscopy approach was pain level, which was assessed five minutes after operation. The assessment was performed on a visual analog scale (VAS) with a horizontal line of 100 mm (0 mm = no pain, 100 mm = intense pain sensation). Every hysteroscopy procedure included documentation of unpleasant or unexpected vasovagal symptoms, such as dizziness and nausea. TENS complications can include skin allergies, pain, or a burning feeling when the electrode is implanted.

After completing the procedure, the level of satisfaction with hysteroscopy was assessed using a verbal Likert scale with the following response options: "very dissatisfied," "dissatisfied," "natural," "satisfied," and "very satisfied."

Results

Table 1 presented the demographic data. In both the active TENS group and placebo group, the mean ages of the patients were found to be comparable ($p = 0.91$), with values of 39.32 ±11.9 and 39.58 ±12.2 years, respectively. The statistical analysis revealed that there were no significant variations in weight and height between the two study groups ($p = 0.44$ and $p = 0.54$, respectively). Moreover, no statistically significant disparity was seen between the groups with regards to parity ($p = 0.64$), as well as the frequency of nulliparous women ($p = 0.51$), gravidity ($p = 0.16$), previous vaginal deliveries ($p = 0.38$), and previous cesarean sections ($p = 0.35$).

In all groups, hysteroscopy was the most commonly requested procedure for the following indications: abnormal uterine bleeding, postmenopausal hemorrhage with suspicious endometrial thickness, and abnormalities observed in ultrasound. After considering any signs of office hysteroscopy or previous office hysteroscopy ($p = 0.53$), there was a non-significant difference between the groups. ($p > 0.05$).

The pain levels during the office hysteroscopy operation were found to be comparable between the TENS and placebo groups. However, no statistically significant differences were observed in the VAS assessment ($p = 0.311$) or pain intensity ($p = 0.11$). Table 2

In relation to the verbal Likert satisfaction rate, it was determined that there was no statistically significant difference in the satisfaction rate between the TENS and placebo groups. ($p = 0.351$). (Table 3)

Both groups reported experiencing symptoms during and after hysteroscopy. There were no statistically significant differences seen in the frequency of vertigo, perspiration, shoulder discomfort, nausea, and dizziness, there were differences with no significance statistically between the groups. (Table 4)

The associations between various factors and VAS scores are presented in Table 5. Positive correlations were seen between the VAS score and the age ($r = 0.256$, $P < 0.001$), as well as parity ($r = 0.404$, $p < 0.001$). Conversely, negative correlations were found between the VAS score and the patients' height ($r = -0.317$, $p < 0.001$), and the Likert verbal scale ($r = -0.278$, $p < 0.001$).

Discussion

Given the extensive utilization of transcutaneous electrical nerve stimulation (TENS) in the management of acute and chronic pain, our objective was to investigate its efficacy in pain reduction during office hysteroscopy and assess patient satisfaction with the operation. Our findings indicate that TENS was not efficacious in alleviating pain during a hysteroscopy, as there was no statistically significant difference observed in the VAS scores (p=0.311) or pain intensity (p=0.11) between the study groups. Furthermore, the present study revealed poor outcomes in relation to pain alleviation through the utilization of TENS, as evidenced by comparable satisfaction rates (as measured by the verbal Likert scale) between the two groups (p = 0.351).

This observation aligns with the findings of a study conducted by Hruby et al. (2010) including 142 patients who were divided into two groups: TENS and controls. The results indicated that the Visual Analog Scale (VAS) scores were comparable before and after the operation, with no statistically significant differences observed between the study groups at time intervals of 30 seconds, 1 minute, and 5 minutes. The study found that the TENS device did not provide any notable alleviation of pain, and the increase in pain scores observed in the control group might be attributable to the higher level of difficulty. This study employed the office cystoscopy method instead of office hysteroscopy.

Nevertheless, our results diverged from those of other research, such as Angelis et al. (11), who assessed the pain level using VAS during office hysteroscopy. The TENS group (A) achieved significant results compared to the control group (B) (group A mean, 3.71 + -2.06; group B mean, 5.07 + -2.03; P <.0004). The observed disparity can be attributed to variations in the utilization of the TNSE device. Specifically, the patients in the TENS group were required to effectively manage their discomfort throughout the hysteroscopy procedure.

During the five to ten minutes preceding the assessment, the patient had the placement of two electrodes in their abdominal region. Due to the device being set to a baseline level of stimulation, the patient experienced a mild tickling sensation in the gap between the electrodes. Subsequently, the patient was instructed on the proper operation of the gadget. If she experienced agony. Lisón et al. (9) conducted a comparison of the VAS scores between persons who received TENS and those in the placebo group. They saw a decrease in pain and discovered the smallest difference that was clinically significant. (P<0.001).

The authors attribute the substantial improvement in pleasure experienced after the surgery to the statistically and clinically significant pain reduction observed in the TENS group. Given this information, it is logical to conclude that the utilization of TENS could enhance the acceptance and frequency of office-based hysteroscopy procedures. The disparity in our study may be attributed to the utilization of an active TENS intervention by Lisón et al. (9), which involved a fluctuating high-frequency (80–100 Hz) and 400-microsecond duration.

However, in a study entitled "Effects of high-frequency, high-intensity transcutaneous electrical nerve stimulation versus intravenous opioids for pain relief after hysteroscopy," Platon et al. (12) conducted a comparison between two distinct methods of pain management after hysteroscopy surgery performed under anesthesia. The researchers found no statistically significant disparities between the two groups when evaluating pain scores exceeding 3. Patients who scored less than 3 on the Visual Analog Scale (VAS) were shown to have a substantially shorter duration of stay in the post-anesthesia care unit (91 minutes compared to 69 minutes, P=0.013) when compared to the opioid responders. He determined that employing TENS as an initial analgesic can reduce the need for postoperative opioids.

The findings of the present study indicate that there were statistically significant connections between the Visual Analog Scale (VAS) of pain and both height and Likert scale ($r = -0.317, -0.278$, and $p < 0.001, 0.001$, respectively). However, a notable positive association was seen between VAS of pain and age. The correlation coefficient is 0.256, with a p-value of 0.001. Lisón et al. (9) found a substantial positive correlation between the measurements of perceived pain obtained from the VAS and Likert scales. The VAS and Likert scale were evaluated at the beginning, contact, biopsy, and five minutes after the procedure. The Spearman rank correlation coefficients were 0.841, 0.890, 0.861, and 0.823, respectively ($P < 0.001$).

The variation in the results can be attributed to the utilization of diverse methodologies for the TENS procedure, variations in pain thresholds among women and cultural backgrounds, or the limited sample size of the study, both in terms of the total findings obtained in this study and those previously reported.

Conclusion

This was a randomized controlled experiment designed to assess the analgesic effects of transcutaneous electrical nerve stimulation (TENS) in office hysteroscopy procedures, as well as patient satisfaction with the intervention. The study's findings show that there was no significant difference in pain reduction and satisfaction levels between people who received transcutaneous electrical nerve stimulation (TENS) and those who received a placebo during hysteroscopy. As a result, we may conclude that the TENS device has no beneficial effect in reducing discomfort during office hysteroscopy. More research is needed to investigate various combinations of analgesic drugs with the goal of reducing pain perception during office hysteroscopy.

Limitation of the study

This research was subject to specific limitations including the cost and availability of TENS equipment in Egypt.

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Competing interest

None to declare.

Consent for publication

Consent for publication was obtained from all participants before their recruitment in the study, and subsequently, following a comprehensive explanation of the study's objectives and methodologies.

Author Contributions

Rania G. El-Skaan: Manuscript writing; Ebtihal S.A. Al-Nomany: data collection and analysis; Mortada E. Ahmed and Hatem H. El-Gamal: Manuscript data revision.

Availability of data and materials

The data that support the findings of this investigation are available on the Data.mendeley.com website, however there are restrictions on their availability because they were utilized under license for the current study and are therefore not publicly available. However, the data are available from the authors upon reasonable request and with permission of the Mendeley data portal from: https://data.mendeley.com/drafts/f9z2vk3brp.

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Figure Legends:

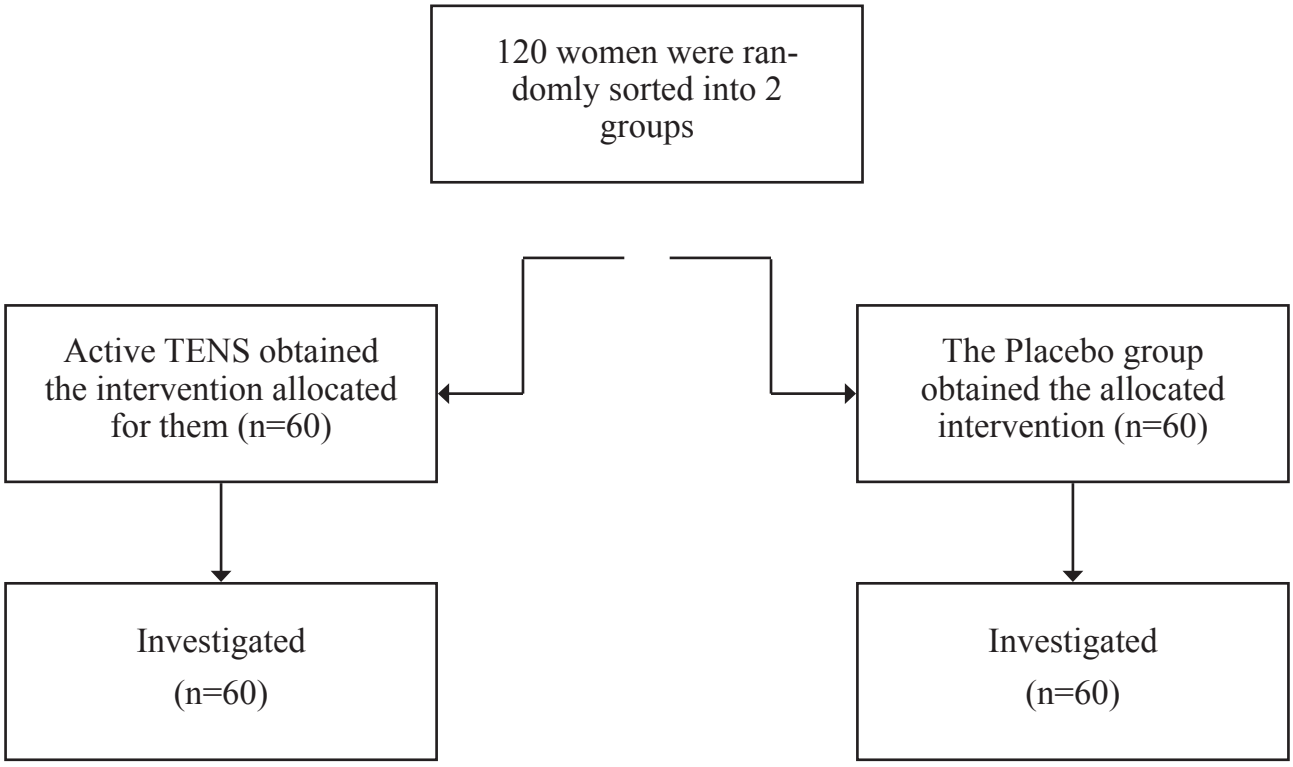

Figure 1. Flow chart.

Table (1): The demographic characteristics, obstetric History, and indications of hysteroscopy of the included patients

Variables	TENS group (N =60)	Placebo group (N =60)	P-value
Age [years]			
- Mean ±SD.	39.32 ±11.9	39.58 ±12.2	0.91
- Median (IQR)	38 (22 -70)	37.5 (21 -74)	
Height in cm			
- Mean ±SD.	158.4 ±5.9	157.7 ±5.8	0.54
- Median (IQR)	160 (150 -170)	158 (150 -170)	
Weight in Kg			
- Mean ±SD.	70.35 ±12.2	72 ±11.4	0.44
- Median (IQR)	70 (50 -98)	70 (50 -98)	
Parity			
- Mean ±SD.	2.3 ±2	2.17 ±2	0.64
- Median (range)	2.1 (0 -7)	2.1 (0 -11)	
Nulliparous, No. (%)	19 (31.7%)	16 (26.7%)	0.51
Gravidity			
- Mean ±SD.	0.5 ±0.9	0.35 ±0.57	0.16
- Median (range)	0 (0 -5)	0 (0 -2)	
Previous Vaginal delivery			
- P0	33 (55%)	40 (66.7%)	0.37
- P1-2	10 (16.7%)	9 (15.0%)	
- P>2	17 (28.3%)	11 (18.3%)	
Previous CS			
- 0	40 (66.7%)	44 (73.3%)	0.52
- 1-2	17 (28.3%)	15 (25%)	
- >2	3 (5%)	1 (1.7%)	

*Data are displayed as mean ±SD, median (Range), or number (%).

Table (2): Pain scores of the included patients

VAS	TENS involved group (N =60)	Placebo involved group (N =60)	P-value
- Mean ±SD.	6.25 ± 3.5	5.6 ± 3.5	0.311
- Median (range)	6 (1 - 11)	7 (0 - 11)	
- No pain	9 (15%)	7 (11.7%)	0.11
- Minimal	5 (8.3%)	6 (10%)	
- Mild	0	5 (8.3%)	
- Moderate	13 (21.7%)	11 (18.3%)	
- Severe	11 (18.3%)	7 (11.7%)	
- Very severe	8 (13.3%)	11 (18.3%)	
- Worst	14 (23.3%)	13 (21.7%)	

Table (3): The participants rate of Satisfaction rates

Verbal Likert scale	TENS involved group (N =60)	Placebo involved group (N =60)	P-value
Satisfaction rate - Very dissatisfied - Dissatisfied - Neutral - Satisfied - Very satisfied	16 (26.7%) 17 (28.3%) 12 (20%) 8 (13.3%) 7 (11.7%)	13 (21.7%) 18 (30%) 10 (16.7%) 12 (20%) 7 (11.7%)	0.351

Table (4): Patients experienced Symptoms.

Variables	TENS group (N =60)	Placebo group (N =60)	P-value
Dizziness, No. (%)	1 (1.7%)	1 (1.7%)	---
Nausea, No. (%)	1 (1.7%)	0	0.5
Shoulder pain, No. (%)	1 (1.7%)	1 (1.7%)	---
Sweating, No. (%)	1 (1.7%)	0	0.5
Vertigo, No. (%)	2 (3.3%)	1 (1.7%)	0.5

Table (5): Correlation between the VAS score and a number of different parameters

	VAS	
	Correlation Coefficient	**P- Value**
Age in years	0.256	<0.001
Weight	0.041	0.56
Height	-0.317	0.001
Parity	0.404	0.001
Satisfaction rate	-.0104	0.134
Likert scale	-0.278	0.001

*Data are presented as a correlation coefficient

DUAL TRIGGER (GNRH AGONIST + HCG) VERSUS HCG TRIGGER in IMPROVING ART OUTCOMES IN POOR RESPONDERS UNDERGOING ANTAGONIST PROTOCOL.

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Running Title

Dual Trigger vs. HCG in Poor Responders

Abstract

Background and aim: Dual triggering, which combines a gonadotropin-releasing hormone (GnRH) agonist and human chorionic gonadotropin (HCG), has been suggested to enhance outcomes in poor ovarian responders undergoing infertility treatment. This study aimed to compare the efficacy of dual trigger versus HCG trigger in improving assisted reproductive technology (ART) outcomes in poor responders using a protocol of GnRH antagonist.

Methods: A prospective clinical randomized trial was conducted at Cairo University from June 2020 to March 2021, involving 86 poor responders. The study dual group (n=43) obtained a dual trigger of 5000 units of HCG plus triptorelin 0.2 mg and, whereas the other group (n=43) was given a conventional HCG trigger. Primary outcomes included the number of retrieved oocytes, mature oocytes (MII), obtained embryos, and fertilization rate.

Results: The study group exhibited significantly higher numbers of retrieved oocytes, MII oocytes, fertilization rate, obtained embryos, and embryos transferred compared to the control group (P value < 0.05). Although the implantation rate and chemical pregnancy rate were higher in the study group, the differences were not statistically significant (P value 0.482 and 0.492, respectively).

Conclusion: dual triggering with HCG and GnRH agonist may enhance ovulation triggering and reproductive outcomes in poor responders undergoing antagonist ICSI cycles, leading to increased numbers of retrieved oocytes, MII oocytes, obtained embryos, and fertilization rate. However, the impact on pregnancy and implantation rates was not statistically significant.

Keywords: Poor responders, Dual trigger, Antagonist cycles, ICSI protocols.

Synopsis: This study compares the effectiveness of dual triggering (using both GnRH agonist and HCG) versus HCG triggering alone in improving ART outcomes for poor ovarian responders undergoing treatment with a GnRH antagonist protocol.

Introduction

Since its inception in 1978, in-vitro fertilization then transfer of embryo has revolutionized infertility therapy, offering hope to many couples (1). Controlled ovarian hyperstimulation (COH) is a critical component of IVF success, enabling the retrieval of multiple healthy oocytes (2). However, a significant challenge in IVF treatment is poor ovarian response (POR), particularly among women over 40, whose numbers seeking fertility treatment continue to rise (3). POR is characterized by suboptimal outcomes in oocyte recovery and pregnancy rates despite various stimulation protocols, including the GnRH antagonist protocol, being attempted (4). One promising approach to improving outcomes in poor responders is "dual triggering," which involves combining a gonadotropin-releasing hormone (GnRH) agonist and human chorionic gonadotropin (HCG) for triggering ovulation (5,6). Dual triggering aims to optimize oocyte development and has shown benefits in normal and high responders, including improved embryo quality and implantation rates, as well as a decreased hazard of stimulation syndrome of ovaries (7,8). In poor responders, dual triggering has shown promise in improving outcomes, even in cases of repetitive immature oocyte retrieval and empty follicle syndrome (9). The purpose of this study is to investigate the potential advantages of dual triggering with a GnRH-agonist and HCG combination on the number of oocytes that retrieved and rate of clinical pregnancy in women with low fertility response during GnRH-antagonist IVF-ICSI cycles.

Patients and Methods

The goal of this prospective clinical randomized experiment was to evaluate and compare the efficacy of a dual trigger (gonadotropin-releasing hormone agonist in addition to human chorionic gonadotropin) to an HCG trigger in women with low fertility response during GnRH-antagonist cycles. The study was conducted at Cairo University Hospital's IVF Unit from June 2020 to March 2021, after scientific and ethical committee permission. The trial population consisted of 86 women with low fertility response to ovarian hyperstimulation drugs undergoing fertility treatment with a GnRH antagonist regimen, who were split into two groups. The experimental group (n=43) got 5000 units of HCG (choriomon®, IBSA 10000 IU) with triptorelin 0.2 mg (Decapeptyl), whereas the control group (n=43) received the typical HCG trigger dosage (10000 units of HCG (choriomon®, IBSA 10000 IU)). The criteria for inclusion included patients who were not responding well to ovarian hyperstimulation drugs during ICSI using a GnRH antagonist regimen. These patients were classified as poor ovarian responders (POR) based on the Bologna criteria, meaning they had at least two of the following three characteristics: reduced ovarian reserve, poor ovarian response in the preceding cycle, and partner age of ≥40. Acute male factor infertility, age over 45, BMI > 30 kg/m2, PCO, other metabolic diseases, and abnormalities of the uterine cavity were among the exclusion criteria. All patients had office hysteroscopy, laparoscopy, hysterosalpingography, and general examination in the course of the study. Additionally, day three serum sample for FSH, LH, E2, AMH, prolactin, and TSH measures as well as baseline 2D transvaginal ultrasonography (MINDRAY DP-5) were performed. On the second day of the cycle, ovarian stimulation was initiated with a beginning dosage of 300 IU of human menopausal gonadotropin (HMG; Merional ®, IBSA) and increased to 450 IU. Beginning

on the seventh day after HMG stimulation, a daily dose from cetrerelix (cetrotide) 0.25 mg was administered. Ultrasound was used to track the ovarian response until at least two of the leading follicles had grown to a diameter of 18 mm. Either 10000 HCG units (choriomon ®, IBSA 10000 IU) for the control group or 5000 HCG units (choriomon ®, IBSA 5000 IU) + 0.2 mg of triptorelin (Decapeptyl) for the study group caused the last stages of oocyte maturation. 34 to 36 hours after triggering, oocyte retrieval was carried out under transvaginal ultrasound supervision. On days two or three following fertilization, a fresh embryo transfer was carried out, and luteal phase support taken as a daily progesterone 100 mg IM as well as a micronized progesterone 400 mg vaginally. Serum pregnancy hormoen β-HCG was assessed 14 days following extraction of oocyte, and for all successful pregnancies, luteal support persisted until the tenth week of gestation. Both groups' data included the number of oocytes obtained, MII oocytes, acquired embryos, rate of fertilization, rate of implantation, rate of chemical pregnancy, as well as rate of clinical pregnancy.

By calculating the mean number of oocytes obtained, the sample size was determined. The mean number of oocytes obtained in dual trigger was around 7± 4.6, whereas in HCG trigger it was roughly 2.3± 2.5, as described in a recent publication by Haas et al. (10). Using the student t-test test for independent samples, to reject the null hypothesis with 80% power at the $\alpha = 0.05$ level, we established a minimum sample size of 10 individuals per group. MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020) was used to calculate the sample size.

Statistical analysis

IBM SPSS Statistics version 24 (IBM Corp, Armonk, NY) was used for data collection, tabulation, as well as analysis. Numerical data that was regularly distributed was displayed as mean and standard deviation, whereas skewed data was shown as median and interquartile interval. Numbers and percentages were utilized to represent qualitative data. The unpaired t test was employed to compare normally numerical distributed data. The Mann-Whitney test was utilized to compare nonparametric skewed data. When comparing categorical data, the chi-squared test or, if applicable, Exact Fisher test were employed. A P value < 0.05 indicates statistically significant value.

Results

This prospective clinical randomized experiment involved 86 women with low fertility response to be managed with ovarian stimulation therapy during IVF-ICSI utilizing a GnRH antagonist therapy protocol. The patients were splitted equally into two groups: the study group, consisting of 43 patients who received triptorelin 0.2 mg (Decapeptyl) in plus 5000 units of HCG (choriomon ®, IBSA 10000 IU), and the other group, comprising 43 patients who received the standard HCG trigger dosage. Table 1 showed that there were no significant differences in age, BMI, duration of infertility, primary or secondary infertility, or causes of infertility (anovulation, male factor, unexplained) between the demographic characteristics of the two groups. These findings indicate that the groups were well-matched at the outset of the study. The ultrasonography characteristics and hormonal profiles of each group of patients are detailed in Table 2. Both groups exhibited similar hormonal and ultrasonography profiles, as evidenced by the lack of significant differences in AMH, basal FSH, basal E2, basal LH, AFC, or endometrial thickness at trigger. Table 3 presents the total dose of gonadotropins used and the duration of stimulation for patients in both groups. Similar ovarian stimulation outcomes were suggested by the lack of significant differences in total gonadotropin

dosage and stimulation duration between the groups. When comparing the dual trigger group to the HCG trigger group, the latter showed significantly fewer follicles, retrieved oocytes, M II oocytes, obtained embryos, and transferred embryos in terms of cycle stimulation results, as seen in Table 4. Furthermore, in the dual trigger group, the rate of fertilization was significantly higher. However, there were no significant differences in implantation rate, chemical pregnancy rate, or clinical pregnancy rate between the two groups. Overall, the data suggest that the use of dual trigger (a combination of GnRH agonist and HCG) in poor responders undergoing IVF-ICSI cycles led to improved cycle stimulation outcomes, including a higher number of retrieved oocytes, M II oocytes, obtained embryos, transferred embryos, and fertilization rate. Comparisons between the dual trigger and HCG trigger groups showed no significant differences in implantation rate, chemical pregnancy rate, or clinical pregnancy rate.

Discussion

The concept of replacing hormone of human chorionic gonadotropin with a gonadotropin-releasing hormone agonist to initiate the last growth of oocyte was first proposed by Gonen et al. over two decades ago but did not gain a lot of attention until the clinical application of GnRH antagonist regimens for IVF(11). The golden goal of GnRH-agonist in triggering was to reduce the hazard of excess stimulation of ovaries through GnRH-antagonist of IVF cycles. Surprisingly, no cases of OHSS were recorded in a series of clinical randomized experiments involving high ovarian responders or normal ovarian responders undergoing fresh IVF treatment and transfer of embryo with GnRH-agonist triggering (12). Previous studies have predominantly focused on high and normal responders, with limited investigation into low responders. Dual triggering was developed as a strategy to address the limitations of GnRH-agonist triggering, which was initially implemented to reduce OHSS risk but was associated with inferior pregnancy outcomes, likely due to compromised endometrial receptivity and altered luteal phase function (13). Compared to traditional HCG-triggered cycles, GnRH-agonist triggering has been linked to significantly lower implantation rates and higher miscarriage rates, despite effectively mitigating the risk of OHSS (14). The adverse pregnancy outcomes associated with GnRH-agonist triggering have been credited to impaired Luteal phase efficiency and decreased susceptibility of endometrium. A new Cochrane article discouraged the widespread of GnRH agonists utilization as the most effective trigger for last growth of oocyte in new IVF cycles due to the substantial decrease in rate of ongoing pregnancy and rate of live birth compared to traditional HCG triggers (15). To address these challenges, the concept of a "double trigger utilization," which consisting of a single GnRH-agonist bolus with a reduced dose of HCG hormone at triggering, has been searched in IVF cycles with high ovarian responders. The inclusion of HCG preserved healthy luteal function while significantly reducing the risk of OHSS associated with GnRH-agonist triggering. Studies in high responders have shown that utilizing a dual trigger significantly improves ongoing pregnancy and live birth rates without significantly increasing the hazard of OHSS (16). To improve the likelihood of pregnancy in GnRH-antagonist phases, just one administration of GnRH agonist added to the typical HCG amount for oocyte development has been tested in normal respondents who are not at high danger for OHSS. Whereas statistics on the number of live babies born were not available, research on double triggering in regular responders found considerably higher continued conception rates among the investigated group in comparison to the other group that obtained just the hormone HCG triggering. (17). The "double trigger" approach has also shown promise in treating empty follicle

syndrome (EFS) in patients with a history of low or poor oocyte yield or immature oocytes obtained (18). A study report showen a successful conception obtained with a double trigger in a patient with EFS. Following two unsuccessful oocyte retrievals, a dual trigger of GnRH agonist and HCG resulted in 11 oocytes retrieved in the third IVF cycle, leading to the transfer of two blastocysts and a live birth. The modification of the treatment regimen with dual triggering yielded a favorable outcome (19). Further research is warranted to evaluate the efficacy of dual triggering in improving rate of baby born lived for women with low fertility response in GnRH-antagonist regimen of IVF-ICSI treatment. Our research goaled to investigate the potential of double triggering for last growth of oocyte, which includes mixing only one injection of GnRH agonist with a standard amount of HCG, in improving rate of babies born live for women with low ovarian response in regimen of GnRH-antagonist of IVF-ICSI therapy. In our search, 86 poor responders undergoing IVF-ICSI with a regimen of GnRH antagonist were split into two sections: the study double trigger group obtained 10,000 units of HCG with triptorelin 0.2 mg, while the other group obtained the standard HCG injection. Our results showed that the study double trigger group had a significantly higher mean number of collected follicles, M II follicles, embryos obtained, and transferred embryos compared to the other group, aligning with previous research demonstrating improved cycle outcomes with double triggering (10,20,21).

Conclusion

According to our research, in patients who are poor responders enduring antagonist ICSI cycles, dual stimulation of ovulation with using HCG hormone plus a GnRH agonist injection may increase the number of oocytes obtained, MII oocytes, embryos acquired, and transplanted embryos. Although there seems to be a correlation between dual trigger and increased frequencies of implantation and pregnancy, the findings lacked statistical significance.

Acknowledgments

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Table 1: demographic data of patients of both groups

Variable	Dual trigger (N =40)	HCG trigger (N=41)	P value
Age (year)[a]	41.6 ± 1.6	41.39 ± 1.8	0.942*
BMI (kg/m²)[a]	25.10 ± 2.5	26.35 ± 3.6	0.546*
Duration of infertility (year)[a]	4.87± 2.3	4.92± 2.28	0.919*
Type of infertility[b] • **1ry** • **2ry**	 29 (67.4%) 14 (32.6%)	 30 (69.7%) 13 (30.3%)	0.816#
Causes of infertility[b] • **Anovulation** • **Male factor** • **Unexplained**	 37 (86%) 4 (9.3%) 2 (4.7%)	 36 (83.7%) 4 (9.3%) 3 (7%)	0.898#

[a]Values (continuous quantitative data) are given as mean±SD.

[b]Values Qualitative (categorical) data are given as numbers (percentage).

Kolmogorov–Smirnov test was used to examine the normal data distributional characteristics of all study cases.

*t-test was used for normally distributed continuous quantitative data

#Chi-square test was used for qualitative (categorical) data

P value <0.05 significant

Table 2: Hormonal profile and ultrasound features among patients of both groups

Variable	Dual trigger (N =40)	HCG trigger (N=41)	P value*
AMH (ng/ml)	1 ± 0.1	1.1 ± 0.1	0.852
Basal FSH (IU/L)	12.3 ± 1.6	12.2 ± 1.6	0.855
Basal E2 (pg/ml)	59.5 ± 9	61 ± 8.8	0.450
Basal LH (IU/L)	4.7 ± 0.62	4.75 ± 0.7	0.770
AFC	4.5 ± 0.7	4.4 ± 0.7	0.631
Endometrial thickness on trigger	10.3 ± 1.1	10.1 ± 1.0	0.426

All Values (continuous quantitative data) are given as mean±SD.

Kolmogorov–Smirnov test was used to examine the normal data distributional characteristics of all study cases.

*t-test was used for normally distributed continuous quantitative data

P value <0.05 significant

Table (3): total dose of gonadotropins used for ovarian stimulation and stimulation duration in patients of both groups.

Variable	Dual trigger (N =40)	HCG trigger (N=41)	P value*
Total dose of GN	378.41±64.6	375±63.38	0.805
Stimulation duration	12.5±0.8	12.7±0.9	0.279

Values (continuous quantitative data) are given as mean±SD.

Kolmogorov–Smirnov test was used to examine the normal data distributional characteristics of all study cases.

*t-test was used for normally distributed continuous quantitative data

P value <0.05 significant

Table (4): cycle stimulation outcomes in patients of both groups.

Variable	Dual trigger (N =40)	HCG trigger (N=41)	P value
Number of follicles[a]	7.15±0.69	7±0.75	0.358*
Number of retrieved oocytes[a]	6.2 ± 1.2	5.6 ± 1.1	0.008*
Number of M II oocytes[a]	4.3 ± 1.1	3.6 ± 1.0	0.003*
Number of embryos obtained[a]	3 ± 1	2.2 ± 0.7	0.0001*
Number of embryos transferred[a]	2.5 ± 0.8	1.9 ± 0.5	0.0001*
Fertilization rate %[a]	69.5 ± 14.9	59.8 ± 14.3	0.003*
Implantation rate %	13.1 ± 6.5	12.3 ± 3.7	0.485*
Chemical pregnancy rate %[b] **Yes** **No**	15 (34.8%) 28 (65.2%)	12 (28%) 31 (72%)	0.485#
clinical pregnancy rate %[b] **yes** **no**	10 (23.25%) 33 (76.75%)	8 (18.6%) 35 (81.4%)	0.596#

[a]Values (continuous quantitative data) are given as mean±SD.

[b]Values Qualitative (categorical) data are given as numbers (percentage).

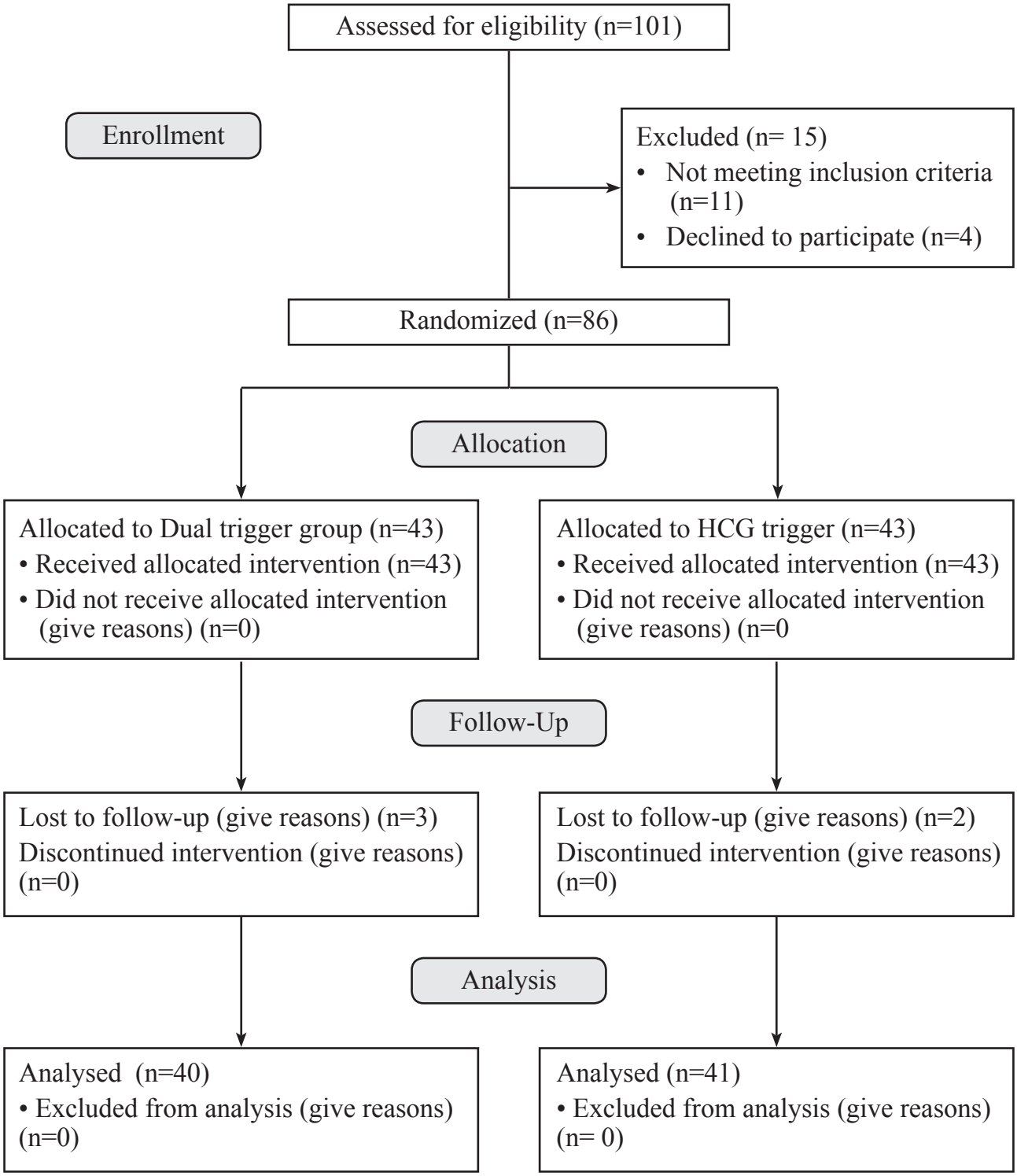
Kolmogorov–Smirnov test was used to examine the normal data distributional characteristics of all study cases.

*t-test was used for normally distributed continuous quantitative data

#Chi-square test was used for qualitative (categorical) data

P value <0.05 significant

CONSORT 2010 Flow Diagram



Effect of Oral Lactoferrin in The Treatment of Bacterial Vaginosis

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Abstract

Background : Bacterial vaginosis (BV) is the most common cause of vaginal discomfort in women. It is characterised by abnormal vaginal microbiota with a depletion of lactobacilli and predominance of anaerobic microorganisms, mainly Gardnerella vaginalis and Atopobium vaginae. Although antibiotics represent an effective therapeutic option in the short-term, recurrent infections still remain a serious problem.

Objectives: This study aimed to characterize the bacterial biota in women affected by bacterial vaginosis (BV) and evaluate the effect of combined orally administered lactoferrin and antibiotic treatment (Metronidazole) versus placebo and antibiotic treatment (Metronidazole) on the vaginal bacterial biota.

Methods: This double-blind randomized controlled clinical trial was conducted at the Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University Maternity Hospitals from December 2021 to December 2023 and included Sixty women diagnosed with BV and randomly divided into two groups: Group A (study group) who received 100 mg of oral lactoferrin plus Metronidazole, and Group B (control group) who received a placebo plus Metronidazole. After excluding seven participants for various reasons, 27 women in Group A and 26 in Group B completed the study.

Results: Both treatment methods were effective for bacterial vaginosis (BV). However, the lactoferrin group showed a significantly greater improvement in clinical signs and symptoms compared to the placebo group after 10 days of treatment. Only 2 participants (7.4%) in the lactoferrin group had vaginal discharge, whereas 8 participants (30.8%) in the placebo group did ($p<0.05$). The cure rate was 85.2% in the lactoferrin group compared to 69.2% in the placebo group, and the recurrence rate was 14.8% in the lactoferrin group versus 30.8% in the placebo group. There were no significant differences between the groups in terms of age, BMI, contraceptive method, history of recurrent BV, sexual activity frequency, baseline clinical signs, symptoms, and vaginal discharge samples.

Conclusions: In conclusion, this study evaluated the effectiveness of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care. The intervention significantly improved BV symptoms, including vaginal discharge, clue cell presence, and fishy odour. However, the differences between the intervention and standard care groups were statistically insignificant. We are aware that no firm conclusions can be drawn about the efficacy of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care from the results derived from such a small-sized study.

Key words: Bacterial Vaginosis, Oral Lactoferrin.

Introduction

Bacterial vaginosis (BV) is a prevalent gynecological condition characterized by an imbalance in the vaginal microbiota, affecting approximately 40-50% of women worldwide (1). The condition is marked by symptoms such as vaginal discharge, odor, and discomfort, significantly impacting the quality of life.(2) The pathogenesis of BV involves a reduction in the population of Lactobacillus species, which are crucial for maintaining a healthy vaginal environment through mechanisms such as lactic acid production, enhancement of the host's innate immune system, and the production of antimicrobial compounds like hydrogen peroxide and bacteriocins (3)

Despite the widespread use of antibiotics, such as Metronidazole, in the treatment of BV, recurrence rates remain alarmingly high (4). Approximately 25% of women experience a second episode of BV within four weeks of treatment, and long-term recurrence rates can exceed 70% (5). The reliance on antibiotics also brings potential downsides, including the development of antibiotic resistance and disruption of the natural vaginal flora. These challenges highlight the urgent need for alternative therapeutic strategies that are both effective and have fewer adverse effects (6).

Lactoferrin, an iron-binding glycoprotein belonging to the transferrin family, has emerged as a promising candidate in this context (7). Produced and stored in secondary neutrophil granules, lactoferrin is released during neutrophil activation and degranulation. It exhibits bacteriostatic and bactericidal properties by binding to iron, thereby limiting its availability to bacteria and inhibiting their growth. Moreover, lactoferrin has immunomodulatory effects and can disrupt bacterial cytoplasmic membranes, enhancing its antimicrobial efficacy. The synergistic effects of lactoferrin when combined with immunoglobulin A, lysozyme, antibiotics, and other drugs further support its potential utility in treating infections (5, 8).

Given these properties, lactoferrin's role in managing BV warrants thorough investigation. This study aims to evaluate the effectiveness of oral lactoferrin combined with Metronidazole in treating BV compared to the standard antibiotic treatment alone. By characterizing the bacterial biota in women affected by BV and assessing clinical outcomes, this research seeks to determine whether lactoferrin can enhance treatment efficacy and reduce recurrence rates.

Patient and method

After ethical committee approval and written consents from the patients, this double-blind randomized controlled clinical trial was performed on a total of Sixty women diagnosed with BV attending the outpatient clinic at Ain Shams University Maternity Hospital in period between December 2021 to December 2023.

Study Population: All included women were randomly (1:1 ratio) allocated to one of the two experimental groups:

- **Group A (study group):** to whom lacto-

ferrin 100 mg (Pravotin® sachets 100mg, Hygint, Egypt) 2 sachets/day for 5 days followed by 1 sachet/day for 10 consecutive days plus antibiotic treatment "metronidazole" 500mg twice daily for 7 days.

- **Group B (control group):**to whom placebo 2 sachets/day for 5 days followed by 1 sachet/day for 10 consecutive days plus antibiotic treatment "metronidazole" 500mg twice daily for 7 days.

Inclusion criteria included sexually active women aged 18-45 years, with a BMI of 18-25 kg/m², regular menstrual cycles, and symptomatic acute BV diagnosed according to Amsel's criteria and Nugent's standardized method of Gram-stain interpretation.

Exclusion criteria included women with medical conditions as diseases like diabetes mellitus led to decrease the immunity and increase the risk of bacterial infection, women with active infections from other pathogens due to change in the vaginal PH and mixed infection can't test the effect of lactoferrin on two modifiable risk factors, pregnant or breastfeeding due to hormonal fluctuations that lead to change in the vaginal environment, vaginal PH and increase the risk of vaginal infection, women who had recent antibiotic or hormone treatments as they lead to decreased immunity and change in the vaginal normal bacterial flora, women with Nugent score <7, women with gynecological conditions causing bleeding as they interfere with the result of vaginal smear and change the vaginal PH and women with allergies to study medications, or refused participation.

Diagnostic criteria of BV:

Amsel's criteria: Amsel et al. (9) described four criteria for the diagnosis of BV in clinical settings. Any combination of three of its four diagnostic criteria (abundant creamy, grey-white adherent vaginal discharge, rotten fish odor - spontaneous or alkali induced -, pH > 4.5, microscopic detected presence of clue cells (exfoliated vaginal epithelial cells covered by G. vaginalis) leads to the diagnosis of BV (9).

In addition to Amsel criteria, BV was also diagnosed by Nugent's standardized method of Gram-stain interpretation in which the Nugent method consisted of microscopic evaluation of bacterial morphotypes to score the overall character of the vaginal flora. Nugent scores range from 0-10, based on the prevalence of 3 bacterial morphotypes that roughly correspond to Lactobacillus, G vaginalis or Bacteroides, and Mobiluncus. The different cell types are counted (Mobiluncus spp., G. vaginalis/Bacteroides spp. And Lactobacillus spp.) and a score between 0 and 10 is obtained; whereby a score of 7–10 corresponds to BV, a score of 4–6 is considered intermediate (partial BV) and a score of 0–3 indicates an undisturbed vaginal microflora. Intermediate scores may indicate the development of BV or a woman that is being cleared of this disease entity; however, these ''intermediate flora'' remains contentious (10).

However, Amsel criteria and Nugent scoring are considered equally efficacious in diagnosing bacterial vaginosis (11).

Sample size Justification:

Group sample sizes of 26 cases per group totaling 52 cases achieve 80% power to reject the null hypothesis of zero effect size when the population effect size is 0.80 and the significance level (alpha) is 0.05 using a two-sided two-sample equal-variance t-test. A 10% excess of cases should be included to compensate for the dropouts, making the total sample size 60 cases divided equally between the two groups

Randomization was done using computer generated randomization sheet.

Allocation and concealment: were done by use of sealed opaque envelopes that were given to a third party (nurse) who assigned the women to study arms. Each woman was invited to pull out an envelope. According to the number inside her envelope, women were allocated to either group 1 or group 2 according to a computer- generated random list.

Detection bias was avoided by blinding patients and assessor to the groups. The sachets in both groups were similar in shape and package and administered anonymously with coding by a midwife colleague with no knowledge of the codes. Final assessment was performed by another colleague who had no information about the groups.

Ethical Considerations: The patient data were anonymous. Data presentation was not be by the patient's name but by diagnosis and patient confidentiality was protected. An informed consent was taken from all participants, it was in Arabic language and confirmed by date and time. confidentiality was preserved by assigning a number to patients initials and only the investigator knew it. Protocol approval by the council of the OB/GYN department at Ain Shams University was done.

Study Procedures:

After a written consent was taken, the recruited women were subjected to a thorough clinical examination that included a full history as well as general, abdominal, and pelvic exams.

History taking: including (personal history, age, obstetric history, medical history, surgical history, past history and family history).

Clinical examinations: include general, abdominal, pelvic exams and assessment of clinical signs and symptoms of vaginosis, and vaginal discharge sampling were performed at each intervention point (base line and after 10 days of treatment).

Investigations: including (labs; CBC, blood group, liver and kidney functions, coagulation profile & imaging; Transvaginal ultrasound).

Sample collection:

Vaginal discharge samples were obtained from the lateral vaginal wall and the posterior vaginal fornix using sterile cotton-tipped swabs. For each participant, two vaginal smears were collected at baseline and after 10 days of treatment.

Vaginal smears were used to assess the BV status: microscopic examination of the fresh smear (detection of clue cells and Gram staining), whiff amine test. Vaginal fluid was measured during each visit using pH test strips.

According to Amsel's criteria, the whiff-amine test was carried out. In detail, the presence of a 'fish odor', attributable to the production of volatile amines, were evaluated by adding a drop of 10% KOH directly to the glass surface, and the presence of fish odor was detected by the researcher's sense of smell. The Amsel's criteria were also performed at the base line and after 10 days of treatment sampling times (9).

According to Nugent scoring system, Lactobacilli are given a score of 0 if found in normal amounts and a numeric score if low in quantity or absent. The other organisms are scored based on numbers present. The sum of the three subscores is the patient's Nugent score: 0-3 is normal, 4-6 is intermediate, and 7-10 indicates bacterial vaginosis (10).

Outcome measures:

- **Primary outcome:** Complete cure rate.
- **Secondary outcomes:** Non recurrence of infection within 4 weeks

Statistical analysis: Analysis is to be performed using SPSS for windows v20.0, Data to be presented in terms of range, mean and standard deviation (for numeric parametric variables); range, median and inter-quartile range (for numeric non-parametric variables); or number and percentage (for categorical variables). Difference between two independent groups is to be analyzed using independent student's t-test as well as the mean difference and its 95% CI (for numeric parametric variables); or chi-squared test as well as the risk ratio and its 95% CI (for categorical variables). Binary logistic regression analysis is to be performed for estimating the association between good/poor response and

the measured variables ROC curves are to be constructed for estimating the validity of measured variables as predictors of good or poor response validity is to be presented in terms of sensitivity, specificity, positive and negative predictive values and their corresponding 95% Cis significance level is set at 0.05.

RESULTS

During this study, 85 patients were assessed for eligibility and 60 patients were included in the study (30 in each group). Of all eligible patients, 16 patients were excluded from the study based on the inclusion criteria and 9 patients refused to participate in of the study. However, three patients dropped out in lactoferrin group and 4 patients dropped in the placebo group during follow-up. Ultimately, the analysis was based on the data of 27 women in lactoferrin group and 26 women in the placebo group.

Table (1): Comparison between study group and control group according to clinical signs and symptoms of vaginosis at baseline and after 10 days of treatment.

Baseline clinical signs and symptoms of vaginosis	Study group (n=27)	Control group (n=26)	Test value	P-value
Vaginal discharge	27 (100.0%)	26 (100.0%)	0.000	1.000
Signs and symptoms of vaginitis after 10 days of treatment				
No signs and symptoms	23 (85.2%)	18 (69.2%)	1.122	0.289
Vaginal discharge	4 (14.8%)	8 (30.8%)		

(t) Student t-test; (p) probability value.

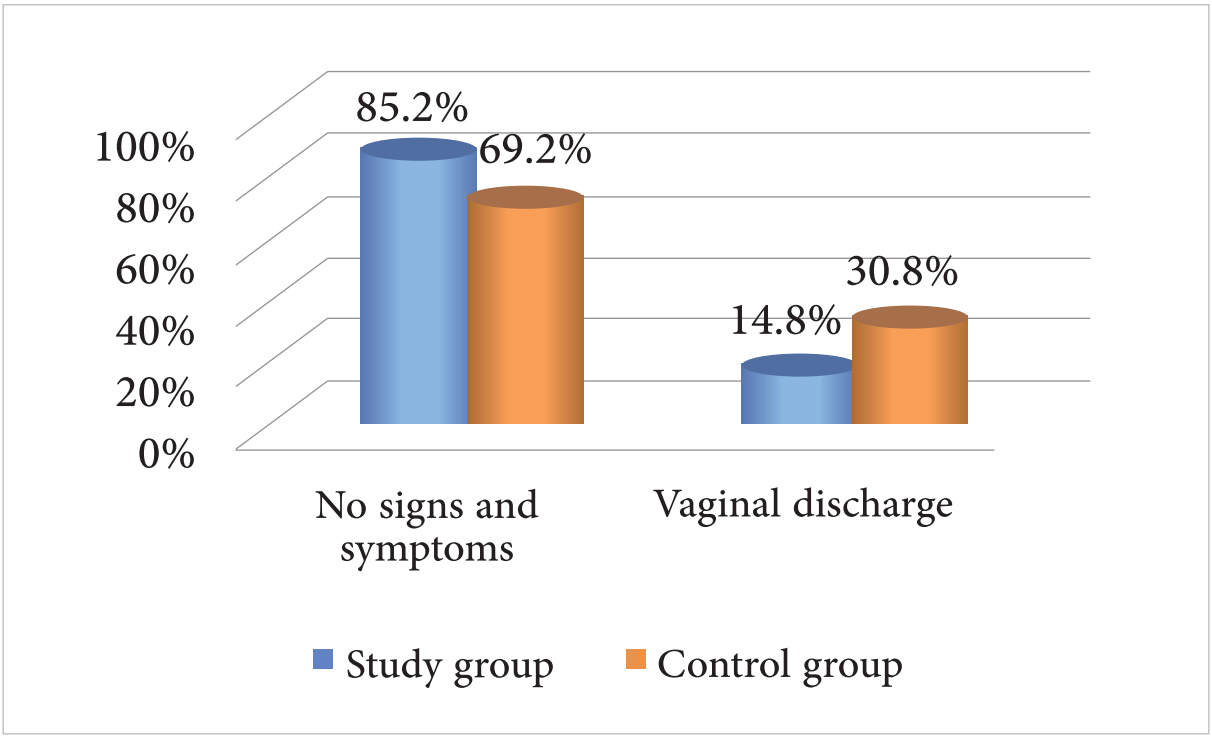

Figure (1): Comparison between Study group and Control group according to Signs and symptoms of vaginitis after 10 days of treatment

Table (1) and Figure (1) show that all studied patients presented with vaginal discharge. However, signs and symptoms after 10 days of treatment were improved compared to baseline in both groups.

Table (2): Comparison between baseline and after 10 days of treatment according to clinical signs and symptoms of vaginosis in the study group.

Clinical Signs And Symptoms of Vaginosis	Study group (n= 27)		t	p
	Baseline	After 10 days of treatment		
No signs and symptoms	0 (0.0%)	23 (85.2%)	13.587	**<0.001***
Vaginal discharge	27 (100.0%)	4 (14.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (2) shows that there was statistically significant higher frequency of improved signs and symptoms after 10 days of treatment in the study group, compared to baseline.

Table (3): Comparison between baseline and after 10 days of treatment according to clinical signs and symptoms of vaginosis in the control group.

Clinical Signs And Symptoms of Vaginosis	Control group (n= 26)		t	p
	Baseline	After 10 days of treatment		
No signs and symptoms	0 (0.0%)	18 (69.2%)	32.8	**<0.001***
Vaginal discharge	26 (100%)	8 (30.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (3) shows that there was statistically significant higher frequency of improved signs and symptoms after 10 days of treatment in the control group, compared to baseline.

Table (4): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the study group.

Vaginal discharge sampling	Study group (n=27)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	2 (7.4%)	15.373	0.002*
pH 5	5 (18.5%)	17 (63.0%)		
pH 6	21 (77.8%)	8 (29.6%)		
pH 7	1 (3.7%)	0 (0.0%)		
pH 8	0 (0.0%)	0 (0.0%)		
Clue cells Baseline				
No clue cells	14 (51.9%)	24 (88.9%)	7.194	0.007*
Positive clue cells	13 (48.1%)	3 (11.1%)		
Fishy Odour Baseline				
Fishy Odour	19 (70.4%)	1 (3.7%)	22.95	<0.001**
No Fishy Odour	8 (29.6%)	26 (96.3%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (4) shows that there was statistically significant reduction mean of pH the after treatment than baseline. Also, there was high frequency of improved fishy odor after treatment compared to baseline with significant reduction in the positive clue cells after treatment comparing to baseline.

Table (5): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the control group.

Vaginal discharge sampling	Control group (n=26)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	1 (3.8%)	15.021	0.005*
pH 5	3 (11.5%)	14 (53.8%)		
pH 6	21 (80.8%)	10 (38.5%)		
pH 7	2 (7.7%)	0 (0.0%)		
pH 8	0 (0.0%)	1 (3.8%)		
Clue cells Baseline				
No clue cells	13 (50.0%)	23 (88.5%)	7.312	0.007*
Positive clue cells	13 (50.0%)	3 (11.5%)		
Fishy Odour Baseline				
Fishy Odour	19 (73.1%)	5 (19.2%)	13.077	0.002*
No Fishy Odour	7 (26.9%)	21 (80.8%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (5) shows that there was statistically significant reduction mean of pH the after treatment than compared to the baseline. Also, there was high frequency of improved fishy odour after treatment comparing to baseline with significant reduction in the positive clue cells after treatment comparing to baseline.

Table (6): Comparison between study group and control group according to outcome.

Outcome	Study group (n=27)	Control group (n=26)	Test value	P-value	Sig
Cured	23 (85.2%)	18 (69.2%)	1.925	0.165	NS
Resistant	**4 (14.8%)**	**8 (30.8%)**			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (6) shows that the higher frequency of cured cases was 23 patients (85.2%) in the study group, compared to 18 patients (69.2%) in control group, with p-value (p>0.05).

Table (7): Comparison between study group and control group according to recurrence of infection within 4 weeks.

Recurrence of infection within 4 weeks	Study group (n=23)	Control group (n=18)	Test value	P-value	Sig
No Recurrence of infection	19 (82.6%)	14 (77.8%)	2.073	0.355	NS
Recurrence after 4 weeks	4 (17.4%)	4 (22.2%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (7) shows that the recurrence of infection after 4 weeks was 4 patients (17.4%) in the study group, compared to 4 patients (22.2%) in the control group, but insignificant difference, with p-value (p>0.05).

DISCUSSION

Currently, the standard-of-care therapeutic approach for BV treatment is based on antibiotic administration, often metronidazole and/or clindamycin (12). Although the antibiotic treatment is effective in about 80% of BV cases, a high recurrence rate (>50%) is reported within 6–12 months. In addition, prolonged and repeated antibiotic treatments facilitate the development of resistant pathogens. In this context, resistance to both metronidazole and clindamycin is well documented among G. vaginalis isolates (13, 14, 15), therefore complementary and/or alternative therapeutic approaches are needed, such as oral lactoferrin. a glycoprotein with antimicrobial and immunomodulatory properties, are being explored.

Since bacterial vaginosis represents major conflict and may be associated with a significant number of obstetric and gynecologic complications, such as preterm labor and delivery, preterm premature rupture of membranes, spontaneous abortion, chorioamnionitis, endometritis, post-Caesarean delivery wound infections, postsurgical infections, and subclinical pelvic inflammatory disease (16), evaluating the efficacy of a probiotic Lactobacillus mixture in combination with bovine lactoferrin as adjuvant to drug therapy with oral metronidazole in adult women with BV was highlighted as a main point of interest (11).

Consequently, this study was conducted and aimed to characterize the bacterial biota in women affected by bacterial vaginosis (BV) and evaluate the effect of combined orally administered lactoferrin and antibiotic treatment (Metronidazole) versus placebo and antibiotic treatment (Metronidazole) on the vaginal bacterial biota.

This double-blind randomized controlled clinical trial was conducted at at Ain Shams University Maternity Hospital (ASUMH) from December 2021 to December 2023 and performed on a total of Sixty women diagnosed with bacterial vaginosis.

The current study revealed that there were no significant demographic differences between the groups, including age, BMI, contraceptive use, infectious disease history and sexual activity among the study groups. This ensured comparability for assessing the intervention's impact on BV.

Regarding the clinical signs and symptoms of bacterial vaginosis, our study results revealed that all patients in both groups presented with vaginal discharge. However, after 10 days of treatment, the clinical signs and symptoms in study group and control group significantly improved and decreased to 14.8% and 30.8%, respectively ***(p value <0.001)***, compared to the baseline with no significant difference between the two group after 10 days of treatment (p value = 0.289).

Regarding the vaginal discharge sampling, our study results revealed that there were no statistically significant differences between study group and control group according to baseline vaginal discharge sampling regarding pH Baseline, Clue cells Baseline and Fishy Odour Baseline, with p-value ($p>0.05$). However, 10 days after treatment, the vaginal discharge sampling revealed significant improvement and reduction of the PH, Clue cells Baseline and Fishy Odour Baseline in both groups with no insignificant difference between groups, with p-value ($p>0.05$).

Regarding the outcome, our study results revealed that 23 patients (85.2%) in the study group were cured, compared to 18 patients (69.2%) in control group, and the recurrence of infection after 4 weeks was 4 patients (17.4%) in the study group, compared to 4 patients (22.2%) in the control group. however, the differences were statistically insignificant, with p-value ($p>0.05$).

It is important to note that vaginal Lactobacillus species can form a protective barrier against pathogen invasion. The metabolites they secrete into the cervicovaginal fluid play a crucial role in inhibiting bacterial and viral infections (17). Additionally, the low vaginal pH (< 4.5) resulting from lactic acid production by Lactobacillus species helps to suppress the growth of pathogenic microorganisms that cause vaginal dysbiosis. The vaginal pH is a key factor in the high incidence of BV among women of reproductive age. Various adjuvant treatments, including ascorbic acid, Lactobacillus strains, and probiotics, have been explored to lower vaginal pH and reduce the recurrence of BV (18, 5).

However, treatment remains unsatisfactory until the pathogenesis of BV is fully understood. Clinicians use different regimens to treat BV, typically involving antibiotics like metronidazole or clindamycin, administered orally or topically. Although many women initially respond to these antibiotics, BV recurs in 11–29% of cases within a month, and an adherent G. vaginalis biofilm often remains post-therapy. Bacteria within biofilms exhibit higher resistance to antibiotics compared to their free-floating counterparts (19).

A promising therapeutic approach involving lactoferrin administration for treating BV has been proposed. Pino et al. (5) conducted an open prospective randomized trial to investigate the bacterial biota of women with BV and evaluate the effects of two different lactoferrin concentrations (100 mg and 200 mg vaginal pessaries) on the composition and dynamics of the vaginal microbiota. The study found that vaginal lactoferrin administration altered the vaginal microbiota in BV patients. Both 100 mg and 200 mg lactoferrin pessaries significantly reduced the presence of bacteria associated with BV, such as Gardnerella, Prevotella, and Lachnospira, while increasing Lactobacillus species. The balance of the bacterial biota was maintained up to 2 weeks after treatment only in women who received the 200 mg lactoferrin pessaries. However, this study is limited by the absence of control group and also, using vaginal route may be effective, compared to the orally administered lactoferrin in our study.

The primary mechanism of lactoferrin's action appears to be its high-affinity iron sequestration (Kd 10–20), creating a bacteriostatic, iron-depleted environment (17). In a later study, Pino et al. (20) evaluated the susceptibility of 71 presumptive Gardnerella vaginalis clinical isolates to metronidazole and clindamycin, and investigated the in vitro antimicrobial activity of Metrodora Therapeutics bovine Lactoferrin (MTbLF) at various concentrations. The study found that lactoferrin's antimicrobial effect was dose-dependent rather than strain-dependent, indicating that the tested strains could use iron for growth. G. vaginalis strains can utilize several iron-containing compounds, including iron salts, hemin, hemoglobin, and human lactoferrin, but not bovine lactoferrin. The inability of bovine lactoferrin to support G. vaginalis growth was confirmed with multiple presumptive clinical isolates.

The specificity of G. vaginalis lactoferrin binding proteins for human lactoferrin suggests that bovine lactoferrin is preferable to recombinant human lactoferrin for BV therapeutics. Additionally, a synergistic effect was observed when MTbLF was used in combination with clindamycin, suggesting that MTbLF could enhance the effectiveness of traditional antimicrobials and help combat antimicrobial resistance.

Furthermore, Russo et al. (11) conducted a double-blind, randomized clinical trial that assessed the efficacy of a probiotic mixture, including Lactobacillus acidophilus GLA-14 and Lactobacillus rhamnosus HN001, combined with bovine lactoferrin, as an adjunct therapy to metronidazole in women with recurrent BV. The study revealed significant improvements in symptoms (vaginal discharge and itching), Nugent scores, and recurrence rates with the probiotic mixture and lactoferrin combination. This alternative approach may represent a safe and effective remedy for restoring healthy vaginal microbiota and preventing recurrent BV.

Lactoferrin may be an effective alternative remedy for preventing BV as it helps restore a healthy vaginal microbiota, predominantly composed of lactobacilli (11). Additionally, Bertuccini et al. (21) demonstrated that bovine lactoferrin, when combined with L. acidophilus GLA-14 and L. rhamnosus HN001, both alone and together, could enhance the biofilm formed by these strains on both abiotic surfaces and HeLa cell monolayers. This promising result supports the potential of a symbiotic mixture of lactoferrin and lactobacilli for promoting vaginal health.

A previous clinical study by De Alberti et al. (22) found that oral administration of Respecta significantly increased vaginal colonization by L. acidophilus GLA-14 and L. rhamnosus HN001 in healthy women after two weeks of treatment. Conversely, the placebo did not result in any increase in lactobacilli levels in the vagina of the treated women. Notably, the levels of both strains continued to rise one week after the cessation of oral intake, suggesting a prolonged persistence of lactobacilli in the vagina.

Extensive research supports the pathogenic role of G. vaginalis and suggests that vaginal Gardnerella spp. biofilm can lead to the ineffectiveness of conventional antibiotic treatments and high recurrence rates, implying that other bacteria within the biofilm may act as opportunistic secondary invaders. Furthermore, bacteria in biofilms exhibit different responses to antibiotics compared to their free-floating (planktonic) forms, with antibiotic resistance believed to be a contributing factor to persistent and recurrent BV. Saunders et al. (23) found that in cases involving BV biofilms, strains of Lactobacillus might be able to disrupt and remove these biofilms, potentially reducing the reliance on antibiotics.

Moreover, lactobacilli supplementation significantly improved symptoms such as discharge and odor, as well as Nugent scores, during the intervention period (24). Anukam et al. (25) conducted a study where, after a 7-day treatment with metronidazole, participants were given either a probiotic oral formulation containing L. rhamnosus GR-1 and L. reuteri RC-14 or a placebo for 30 days. The study reported an initial cure rate of 88% for the probiotic group compared to 40% for the placebo group ($P<0.001$), with a significant increase in lactobacilli in vaginal swabs from the treated group. However, due to the limited amount of data, probiotics cannot yet be recommended for women with BV.

The benefits of using probiotics should be evaluated alongside known risk factors for BV, such as diet, douching, contraception type, and menstrual cycle. To sustain and maximize long-term benefits, BV should be managed as a chronic condition requiring both short-term and long-term treatments (19). The unclear causes of BV, high recurrence rates, sub-optimal treatment options, often inconsistent clinical advice, and distressing symptoms remain significant chal-

lenges in modern obstetrics and gynecology (26). Consequently, it is not surprising that women often resort to various self-help remedies to prevent further recurrences, and doctors are continually searching for new therapeutic strategies.

The differences between these studies and ours might be attributed to the use of a probiotic mixture, including Lactobacillus acidophilus GLA-14 and Lactobacillus rhamnosus HN001 combined with bovine lactoferrin (Respecta), the prolonged treatment period, and the different methodology. In the study by Russo et al. (11), Respecta or placebo was administered as one capsule per day for 10 consecutive days, repeated each month during a six-month follow-up period, starting on the first day of the menstrual cycle. This approach considers that menstrual blood increases vaginal pH and thereby heightens the risk of recurrences.

Although many healthcare providers might view vulvovaginal infections as relatively straightforward to treat, treatment failures and recurrent infections are common. Patients frequently receive repeated courses of the same ineffective treatments. However, for most women experiencing chronic or recurrent vaginal infections, there are evaluation and treatment approaches that can provide more satisfactory outcomes. In this context, the use of probiotics may be a viable therapeutic strategy (11).

These findings may underscore the potential of lactoferrin in managing BV, highlighting its role in reducing clinical signs and symptoms, particularly vaginal discharge. However, some variability in treatment response suggests the need for personalized approaches and further research to optimize treatment strategies.

The strength points of this study:

The strength points of this study are that it is double-blind randomized controlled clinical trial design, its setting at a single tertiary care center and presence compared control group. To the best of our knowledge, there is a paucity of studies in literature evaluating the bacterial vaginosis treatment with lactoferrin, compared with control group, and that represents a strength point of our study.

The limitations of the study

The findings of this study should be interpreted in light of its limitations firstly, including relatively smaller sample size relative to the previous studies, not being a multicentric study and this represents a significant risk of publication bias. Secondly, using only orally administrated route and small dose of the drug (lactoferrin 100 mg) which may underestimate the antimicrobial effect of Lactoferrin as the antimicrobial effect of Lactoferrin was dose-dependent and not strain-dependent (Pino et al., 2022). Thirdly, short period of treatment and follow up.

Conclusions

In conclusion, this study evaluated the effectiveness of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care. The intervention significantly improved BV symptoms, including vaginal discharge, clue cell presence, and fishy odour. However, the differences between the intervention and standard care groups were statistically insignificant.

We are aware that no firm conclusions can be drawn about the efficacy of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care from the results derived from such a small-sized study. Nevertheless, the present study can burden the knowledge and shed some light on future prospective studies with larger sample sizes, high dose of lactoferrin mixed with probiotics and several lactobacilli strains with long period of treatment and follow up demonstrating the long-term effect of high dose of lactoferrin.

Conflict of interest

the candidate declared that there is no conflict of interest and the cost of the study was paid by the candidate.

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